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CONTENTS

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CANCER RESEARCH

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The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain

III. Observations on Adrenal Glands and Accessory Sex Organs in Mice 13 to 24 Months of Age*

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Gonadectomized mice of the ce strain are of interest because they have a high incidence of adrenal cortical carcinomas (2). These tumors are extremely rare in normal mice, as evidenced by observations at the Jackson Memorial Laboratory and by the infrequent references to such growths in the literature. Adrenal cortical carcinomas are themselves of special interest because of evidence that they are the source of internal secretions of the nature of sex hormones (3).

This is a report of observations on ovariectomized and intact ce female mice from 13 to 24 months of age. In earlier reports ovariectomized mice of this strain up to 1 year of age were discussed.

MATERIALS AND METHODS

The material described in Part I (2) was used, with the exception that the ages of the animals at autopsy ranged from 13 to 24 months rather than from 1 to 12 months. There were 15 unspayed and 41 ovariectomized virgin females in this age group. The method of study has been described (2, 3).

RESULTS

Frequency of adrenal tumors.—Adrenal tumors were present in all 41 ovariectomized animals (Table I); in 30 they were bilateral. The left adrenal was involved in 38 mice and the right in 33. The growths were larger on the left side in 23 and on the right side in 15 mice; in 3 no comparison was made. No adrenal cortical tumors were present in 15 intact mice of the same strain and ages.

Increase in size of adrenal tumors.—The growth of many of the adrenal tumors was estimated in a general way by palpating the tumor sites at intervals of 4 or 8 weeks during the later months of life. The results with 1 mouse (P2511) were as follows: No tumor felt at 11 months, small growth at 12 months, medium at 15 months, medium to large at 16 months, and large at 18 months (autopsy age). In some cases a large carcinoma could be sustained for several months, as in P2524, where the neoplasm was medium to large at 12 months, large at 16 months, and large at 19 months (autopsy age), and on the whole these tumors grew over a period of several months without causing death. Indeed, many of the animals died with large adrenal growths between the ages of 16 and 18 months. Few of the ovariectomized mice lived more than 18 months.

Histology of adrenal tumors.—The histology of all the adrenal tumors in this series except those in 6 animals has already been described (1, Fig. 6). Brief descriptions of these 6 follow.

P2794 was ovariectomized at birth and autopsied when 15 months of age. An adrenal cortical tumor on the left side had the following dimensions: cephalic-caudal diameter, 13.5 mm.; lateral, 13.5 mm.; dorso-ventral, 12 mm. Microscopic examination showed that the tumor had maintained itself in fairly good condition throughout. The major part was composed of diffusely arranged cells, the lesser portion of smaller cells with very darkly staining nuclei; the latter were typically separated into strands by connective tissue to form a distinct pattern. Within open spaces among

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these small cells lay small groups of large cells with lightly staining nuclei and brownish cytoplasm that contained many small vacuoles. The right adrenal contained a round tumor, 6 mm. in diameter, which was separated into two rather distinct parts by cell types similar to those in the left adrenal growth.

P2793, autopsied at 20 months of age, had a neoplasm in the left adrenal with the following diameters: cephalic-caudal, 20 mm.; lateral, 20 mm.; dorsoventral, 15 mm. The tumor was composed of the cell types that occurred in P2794, but with one addition. Deep within the growth there were small, sharply limited areas that contained large polygonal cells with lightly staining clear cytoplasm and deeply staining, round nuclei. Unusual tumor cells resembling those described for P2466 (1) were present in another small area. The right adrenal was free of tumor.

P2792, autopsied when 20 months of age, had a tumor of the left adrenal with the following diameters: cephalic-caudal, 27 mm.; lateral, 22.5 mm.; dorsoventral, 18.5 mm. This neoplasm was similar to the tumor of the left adrenal in P2794, except that extensive areas showed degenerative changes. The right adrenal contained a small growth, 2 mm. in diameter and similar to the smaller neoplasm in P2794. A large amount of normal cortex and medulla partly surrounded the tumor.

P2780, autopsied at 22 months of age, had a tumor of the left adrenal measuring 20 mm. in the cephalic-caudal diameter, 20 mm. in the lateral, and 26 mm. in the dorsoventral. The growth was similar in structure to the larger tumor in P2794.

P2556, autopsied when 23 months of age, had a tumor of the right adrenal that was nearly round, measured 15 mm. in diameter, and was similar in structure to the larger tumor in P2794. The left adrenal was the site of a minute growth that contained only the small type of cell.

P2781, autopsied when 24 months of age, had a tumor of the left adrenal with a cephalic-caudal diameter of 19 mm., a lateral of 13 mm., and a dorsoventral of 12 mm. The structure was similar to that of the larger tumor in P2794. The right adrenal contained 2 minute growths composed entirely of cells of the small type.

Metastases.—Metastases from these adrenal neoplasms were found in the lungs in 9 mice (Table II). The 2 different cell types described were found in 3 cases, only cells of the small, darkly staining type in 2, and only cells of the large type in 4; several of the metastastic tumors were lobed. The pulmonary metastases in P2792 are shown in Fig. 4. Secondary growths in other organs were not noted.

Submaxillary gland.—In all the intact controls the submaxillary gland was of the female type, whereas in the ovariectomized mice it was usually of the male type (Fig. 7). An exception was P2523, 16 months of age, in which this gland was indifferent in type. This mouse had very small adrenal tumors and little evidence of sex stimulation, as shown by careful examination of the accessory sex organs; for example, the lining of the vagina was 2 cell layers thick as in the sexually immature mouse. P2513, 17 months of age, had a submaxillary gland that closely approximated the female type (Fig. 8); this mouse had an unusual

TABLE I: SIDE OF OCCURRENCE OF ADRENAL TUMOR, AND SIZE IN RELATION TO SIDE

Mouse number	Age at autopsy, months	Adrenal tumor left side = + larger on left side = ++	Adrenal tumor right side = + larger on right side = ++
P2297	13	+*	+*
P2308	13	+	++
P2419	14	++	+
P2318	14	++	+
P2449	15	++	--
P2794	15	++	+
P2466	15	++	—
P1656	16	+*	+*
P2396	16	+*	+*
P2523	16	+	++
P2521	16	++	+
P2522	16	++	+
P1660	17	++	+
P2450	17	++	+
P2671	17	++	—
P2512	17	+	++
P2513	17	+	++
P2719	17	+	++
P1699	17	++	+
P2692	17	++	—
P2691	18	+	++
P2506	18	—	++
P2511	18	++	+
P2510	18	++	+
P2773	18	+	++
P2542	18	+	++
P2519	19	+	++
P2527	19	++	+
P2524	19	++	+
P2526	20	+	++
P2793	20	++	—
P2572	20	—	++
P2792	20	++	+
P2741	20	+	++
P2560	22	—	++
P2585	22	++	+
P2583	22	++	—
P2579	22	++	—
P2780	22	++	—
P2556	23	+	++
P2781	24	++	+
		Total: 38 tumors on left side. 23 tumors, larger on left side.	Total: 33 tumors on right side. 15 tumors, larger on right side.

* Side not known.

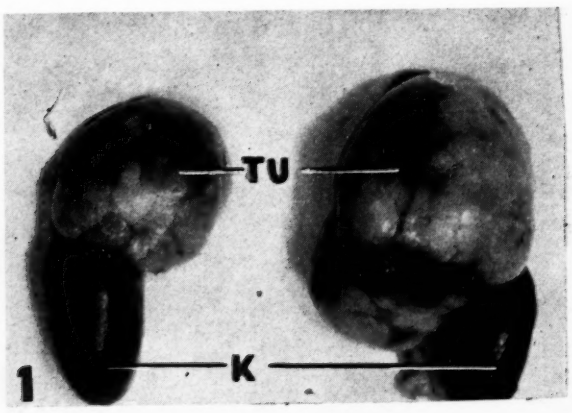
TABLE II: OBSERVATIONS ON ADRENAL TUMORS, ACCESSORY SEX ORGANS, ETC.

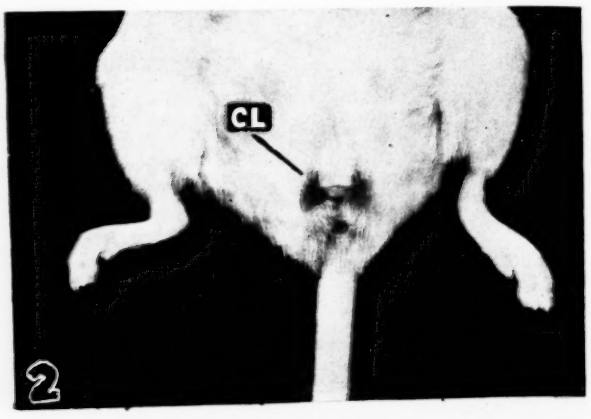
Mouse number	Tumor*	Sub-maxillary gland	Uterus, diameter	Vaginal epithelium, number of cells thick	Mammary glands			Body weight, gm.
					Evidence of duct development	End buds	Alveoli	
P2297	small	♂-type	medium	5–6	yes	no	no	
P2308	large	"	large	10–14	"	"	"	
P2419†	"	"	medium	6–10	"	"	"	
P2318	"	"	large	12–18	"	"	few	
P2449	"	"	"	—	"	"	no	
P2794	"	"	"	5–6	"	"	"	34
P2466	"	"	"	5–6	"	"	"	
P1656†	"	—	"	3–4	"	"	"	
P2396	"	♂-type	medium	6–8	"	"	"	
P2523	small	indifferent	small	2	slight	"	"	
P2521†	medium	♂-type	medium	10–12	"	"	"	
P2522	large	"	large	6–8	yes	"	"	
P1660	medium	"	"	6–8	"	"	"	
P2450	large	"	"	—	"	"	yes	
P2671	medium	"	medium	2–3	slight	"	no	24
P2512	large	"	large	2–3	yes	"	yes	
P2513	small	♀-type	"	6–8	"	"	no	
P2719	large	♂-type	"	2	"	"	yes	34
P1699	medium	"	"	2	slight	"	no	
P2691	large	"	"	10–12	yes	"	"	30
P2692	"	"	medium	2–3	slight	"	"	30
P2506†	"	"	"	2	—	—	—	
P2511	"	"	large	—	yes	no	a few	
P2510	"	"	"	—	"	"	no	
P2773	"	"	"	2	"	"	yes	38
P2542	"	"	medium	2	slight	"	no	
P2519	"	"	large	6–8	yes	"	"	
P2527	"	"	small	2	slight	"	"	
P2524†	"	"	large	2	yes	"	"	
P2526†	"	"	"	2	slight	"	"	
P2793†	"	"	"	2	yes	"	"	35
P2572	"	"	small	2	slight	"	"	
P2792†	"	"	large	2–3	yes	"	yes	40
P2741	"	"	"	2–3	"	"	"	31
P2560	"	"	"	4–6	"	"	"	
P2585	"	"	"	2	slight	"	no	
P2583	"	"	"	2	"	"	"	
P2579†	medium	"	medium	2	"	"	"	
P2780	large	"	large	2	"	"	"	34
P2556	"	"	"	5–6	yes	"	yes	
P2781	"	"	medium	2	slight	"	no	32

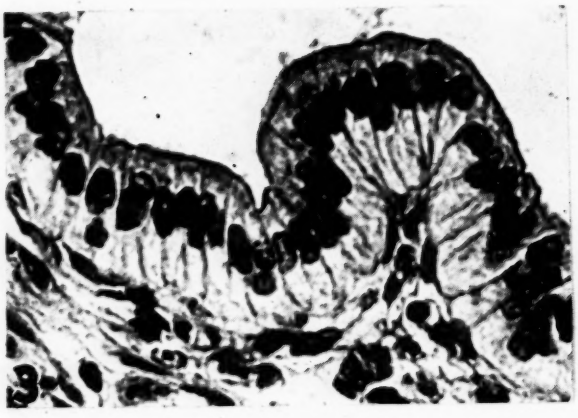
*Under 0.5 cm. in diameter = small; 0.5 to 1.5 cm. in diameter = medium; more than 1.5 cm. in diameter = large.
†Metastasis of adrenal tumor to the lung.

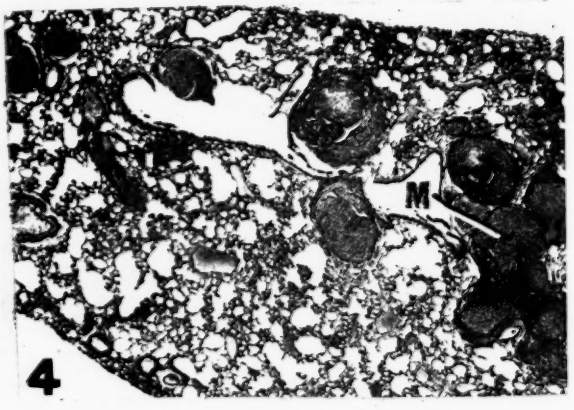
TABLE III: NUMBER OF INTACT MICE AUTOPSIED AT DIFFERENT AGES, WITH OBSERVATIONS ON VARIOUS ORGANS, ETC.

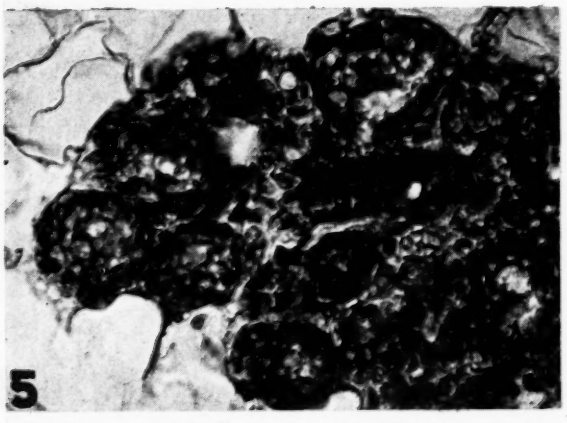
Age at autopsy, months	Number of mice	Adrenal cortical tumors	Sub-maxillary glands	Uterus and vagina	Mammary glands			Average body weight, gm.
					Ducts	End buds	Alveoli	
13	1	none	♀-type	normal	normal	none	none	27.0
15	1	"	"	"	"	"	"	27.0
16	1	"	"	"	"	"	"	28.0
18	4	"	"	"	"	"	"	28.7
19	1	"	"	"	"	"	"	29.0
20	1	"	"	"	"	"	"	28.0
21	3	"	"	"	"	"	"	25.0
22	1	"	"	"	"	"	"	27.0
23	1	"	"	"	"	"	"	26.0
24	1	"	"	"	"	"	"	28.0

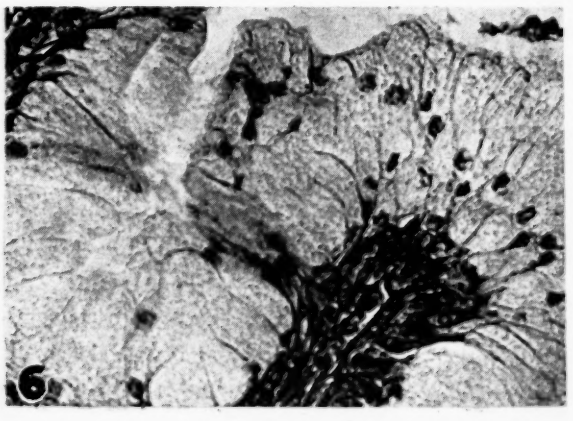


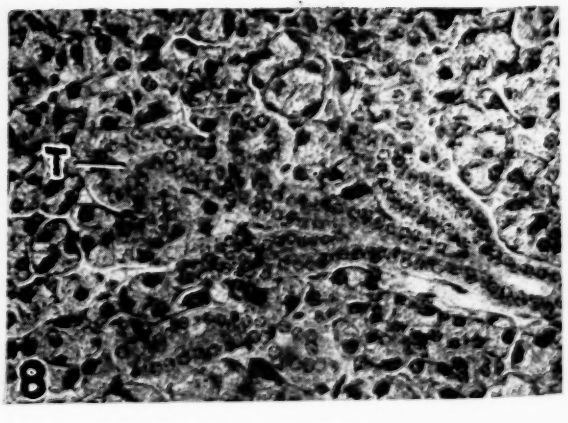












FIGS. 1–8

type of adrenal tumor, described in an earlier paper (1).

Uterus.—In the intact controls the uterus remained large in diameter up to 24 months. Metaplasia of parts of the uterine epithelium was present in P2681, P2709, and P2753 and was extensive in P2653. The uterine glands were cystic in P2768 and P2655.

In the ovariectomized mice the uterus was frequently as large in diameter as, and in some cases slightly larger than, in intact females. Its diameter varied much more from one mouse to another in the ovariectomized than in the intact group. The size of this organ seemed to be correlated in general with the size of the adrenal tumor; thus P2523, with a very small growth, had a uterus of very small diameter, while mice with larger neoplasms generally had larger uteri. However, the correlation was not close in all cases, as P2572 and P2527, with large adrenal tumors, had uteri only about one-third the diameter of some other mice with large adrenal growths. The size of the uterus is indicated for each mouse in Table II.

Eleven mice had cystic uterine glands, and variations in the height of the epithelial cells lining the lumen were noted. In 10 of these animals the nucleus was centrally located in medium to tall columnar cells (Fig. 3) and in 2 at the lumenal end. In the others, and in the intact controls, the nuclei occupied a basal position. A lumenal position of the nucleus has been reported present at the time of the embedding of ova.

Vagina.—The vaginal epithelium in unspayed virgin mice represented various stages of the estrous cycle, which in some cases may have been continued into advanced life, as P2748 had an epithelial layer 10 to 12 cells thick at 21 months, with a stratum corneum in addition.

The vaginal epithelium in the ovariectomized mice was characteristically thinner than in the intact controls, or in ovariectomized ce mice at a slightly earlier age. However, in P2691, 18 months of age, the stratum germinativum was 10 to 12 cell layers thick. The stratum corneum was intact in some places, while in others it had desquamated and the then superficial layer was heavily infiltrated with leukocytes. In most mice 17 months of age or older the vaginal epithelium was 2 cell layers thick and had undergone extensive mucification (Fig. 6).

Cyclic behavior of vaginal epithelium.—The cyclic behavior of the vaginal epithelium in ovariectomized mice was examined in 5 such animals that were just over 1 year of age, by determining the percentage of leukocytes and of nucleated and cornified epithelial cells in smears taken daily for 26 days.

P2671 gave evidence of an estrous period 10 days after the beginning of the study and again 12 days later, and an adrenal tumor somewhat larger in diameter than the kidney was discovered by palpation. P2691 had only 1 estrous period in 26 days; an adrenal tumor slightly smaller than that in P2671 was found. P2741 had 3 periods of estrus in 26 days and a growth similar to that in P2671 was present. P2780 had no cycle and no palpable tumor. P2781 had a high percentage of cornified cells, indicating at least an attempt at estrus, on the 13th day and again 11 days later, but no adrenal neoplasm was found on palpation.

Vaginal smears made on a group of ovariectomized dba mice of similar age at the same time as the study described above gave evidence that the cyclic behavior of the vaginal epithelium is more pronounced and occurs at shorter intervals in this than in the ce strain.

Pituitary.—In the intact controls no abnormalities of the pituitary were discovered. In the ovariectomized mice a few irregularities were noted upon gross examination, but without differential staining little information could be obtained of their nature. Five such were found in mice 17 months of age or older (P2719, P2773, P2792, P2560, and P2556) but none under that age.

Clitoris and clitoridean glands.—The clitoris was definitely enlarged in only one case; an 18 month old ovariectomized mouse, P2511 (Fig. 2), in which the change was noted at least 2 months before autopsy.

The clitoridean glands were small in all the intact mice, but distinctly enlarged in a number of ovariectomized mice 18 months of age or older (P2511, P2542, P2527, P2526, P2524, P2741, P2560, and P2780). Three of these (P2741, P2560, and P2780) had glands at least half the size of the preputial gland in intact males.

DESCRIPTION OF FIGS. 1 TO 8

FIG. 1.—Adrenal tumors of ovariectomized ce female P2524 when mouse was autopsied at 19 months of age. Ventral view. Tu, tumor. K, kidney.

FIG. 2.—CL, enlarged clitoris of P2511 at 18 months of age.

FIG. 3.—Epithelial lining of uterine gland showing lumenal position of nuclei. P2542, 19 months of age. Mag. × 650.

FIG. 4.—Nodules of adrenal tumor in lung of P2792 at 20 months of age. M, one of numerous areas with adrenal tumor tissue.

FIG. 5.—P2719, 17 months of age. Mammary gland, showing clumps of alveoli packed along a duct.

FIG. 6.—P2792, 20 months of age, showing mucification of vaginal epithelium. Mag. × 360.

FIG. 7.—Male type submaxillary gland of P2511 at 18 months of age. T, terminal tubule. Mag. × 260.

FIG. 8.—Female type submaxillary gland of P2513 at 17 months of age. T, terminal tubule. Mag. × 260.

Mammary glands.—In the unspayed virgin mice the mammary glands were well developed as far as main ducts, branches, and twigs were concerned, and persisted with little evidence of regression up to 24 months. As a standard for comparison it may be said that the duct system in the ce strain is not so extensive as in dba virgin females. The degree of development from gland to gland within individual mice was considered uniform. End buds, which form at the approach of estrus in the young mouse, were not found, and alveoli, which normally form during pregnancy, were not observed. If the mamma be considered as a compound tubulo-alveolar gland, it may be said that the tubular parts were developed whereas the alveolar were not. There were no localized irregularities of growth such as have been termed precancerous lesions. No mammary carcinomas appeared.

Although normal uniformity existed from one mammary gland to another within individual ovariectomized mice, great variation was present from one mouse to another.

Some of the differences in the growth of the mammary glands were differences in degree of duct extension, width, and subdivision. Others involved alveoli as, for example, whether these were present or absent, and, if present, whether or not they were extensively developed.

Since the mammary gland is largely dependent on the products of the glands of internal secretion for its development, it would seem that there must have been great variations in the amount or/and nature of these secretions.

In some mice the mammary glands consisted of only a few short, slender primary ducts and their branches, while in others the ducts were long, medium wide, and extensively branched and sub-branched. The degree of development is indicated in Table II. To a certain degree the duct development was related to the size of the adrenal tumor; that is to say, very small growths were always associated with very slight mammary development. Not all the larger neoplasms, however, were associated with extensive duct growth. For example, P2585, at 22 months, had a large amount of tumor distributed in both adrenals, but little mammary gland development; on the other hand, P2524, with adrenal growths of similar size and distribution, had extensive mammary development.

A fairly sharp distinction was possible when considering whether or not alveolar development had occurred. Absent in virgin females, this was present and attained a high degree in some of the ovariectomized mice (Fig. 5). The degree varied greatly from mouse to mouse, but not from gland to gland in the same animal. Alveoli were present in 11 mice, in the youngest before 13 months (2) and in the oldest at 23 months. In all cases where alveoli existed there was a large amount of adrenal cortical tumor. However, in a number of mice where there was a large amount of neoplastic tissue alveolar development did not take place. All mice in which it did occur had submaxillary glands that were definitely of the male type; but again, all mice with such submaxillary glands did not show alveolar growth.

One condition that seemed to be correlated to some extent with alveolar proliferation was abnormality of growth in the pituitary gland; in the 5 mice with pituitary tumors that were evident upon gross examination alveolar development had occurred, and in no case were abnormalities of the pituitary noted without alveolar growth. This relationship should be subjected to more critical observation.

Neither localized irregularities of growth, the so-called precancerous lesions, nor established tumors were found in the mammary glands of the ovariectomized mice.

Exceptional mice.—P1147, ovariectomized at birth and autopsied when 14 months of age, was considered an exceptional type of mouse and therefore is not included in the general series. She had 2 adrenal cortical tumors; one on the left side 1.5 cm. in diameter, and a much smaller one on the right side. In addition, material that was interpreted on gross examination as ovarian tissue was saved from the region in which an ovary would normally be present. Under the microscope it proved to be an ovarian tumor similar in structure to the adrenal neoplasms. A small amount of tissue resembling ovary and containing follicles was present at one edge of this growth.

The submaxillary glands had not been saved for study. The uterus was large in diameter and had medium high epithelial cells with clear cytoplasm and oval nuclei in the basal position. These cells were in close contact with the stroma; the basement membrane was not prominent. The cells of the stroma seemed more or less shrunken; their nuclei were not vesicular. The pituitary was slightly enlarged, and there were numerous blood-filled areas in the anterior lobe. The vaginal epithelium was 5 to 6 cell layers thick, with a few leukocytes in the outer edge. The cells stained more darkly in the basal region than in the upper part of the epithelium. The condition of the vagina resembled that of a stage in metestrus.

P2418, ovariectomized at birth and autopsied at 16 months of age, is not included in the general series. No adrenal cortical carcinomas were found at autopsy, though minute growths interpreted as small benign tumors were present in the outer edge of one cortex. A tumor 1 cm. in diameter, discovered near the site from which an ovary had been removed, was very similar in structure to the adrenal cortical tumors.

The submaxillary gland had moderately tall columnar cells, with basal nuclei, in the terminal tubules.

The tubules did not occupy so great a part of the total area of the gland as in normal male mice, and the gland as a whole was considered intermediate between the male and female types. The pituitary was normal in size. The vagina had not been saved for study. The uterus was of moderate size with stromal cells closely packed and nuclei distinct. The uterine glands were small; the lumen was of the simple slit type. The epithelial cells lining the lumen were low, with oval nuclei at the base. There was a clear area between the bases of these cells and the stroma.

Nonmasculinized mouse P2513.—This mouse, autopsied when 17 months of age, was the only ovariectomized mouse in this series with typically female submaxillary glands. The right adrenal contained a small cortical tumor, approximately 3×1.5 mm., and in addition an unusual growth about one-half as large with structures reminiscent of the ovary. The left adrenal contained 2 minute cortical tumors in addition to a lesion that was interpreted as precancerous.

The uterus was large in diameter, with a branched lumen lined by moderately tall, closely packed cells with oval nuclei situated near the base. There was no clear zone between these cells and the stroma. The stromal cells were shrunken and had small nuclei. Capillaries were distributed through the stroma. The uterine glands were large, but not cystic.

In certain regions the vaginal epithelium was 6 to 8 cell layers thick, with basal cells that stained much darker than the more superficial ones. The lumen was free of leukocytes, but the superficial epithelium had vacuoles in which leukocytes had degenerated. In other regions of the vagina the epithelial layer was 2 to 3 cell layers thick and lined with mucous cells; mucus was present in the lumen near these cells.

The pituitary was normal.

The mammary ducts were moderately long with many main and collateral branches. Alveoli were not present. The glands were similar to those in virgin females of the ce strain at a comparable age.

DISCUSSION

When the 75 ovariectomized and 41 intact ce mice autopsied at 1 to 24 months of age are considered as a whole, a number of interesting conditions emerge. Two of the most general are (a) that ovariectomy was definitely related to the occurrence of adrenal carcinoma; 100 per cent of the ovariectomized mice 6 months of age or older had such a growth in one adrenal, whereas none were found in intact mice of any age; and (b) that in all mice where moderate or large amounts of adrenal cortical tumor were present, the accessory sex organs were developed. The degree and nature of this development varied from mouse to mouse, as though there were differences in the type of internal secretions or in the proportions of the several different ones.

In comparing the present results with those in mice less than 1 year of age it was found that in both there was a progressive change of the accessory sex organs as age advanced. This was evidenced in a number of ways; in the older, or present group, for instance, by increased alveolar development in the mammary glands and by the condition of the uterine and vaginal epithelium.

The explanations are only speculative. It may well have been that the size of the adrenal tumor and the resulting poor health of the host was one factor, which possibly caused a reduced estrogen level and increased vaginal mucification. Another may have been the result of long continued interaction between the tumor in the adrenal and the other glands of internal secretion; probably between the tumor and the pituitary at least. The latter may have been responsible for the increased alveolar development in the mammary glands.

Further experimentation will be necessary to determine the mechanism responsible for variations between strains following ovariectomy. The solution of this problem may help to explain the presence or absence of certain ovarian tumors (2).

SUMMARY

Two groups of mice of the ce strain, 13 to 24 months of age, were autopsied at monthly intervals and studied. The groups consisted of: (a) 15 unspayed virgin females, and (b) 41 gonadectomized virgin females. Ovariectomy was performed when the mice were 1 to 3 days of age. Adrenal cortical carcinomas were found in 100 per cent of the ovariectomized mice and in none of the intact. Metastases to the lungs were observed in 9 mice. The condition of the accessory sex organs of the ovariectomized mice indicated that they were being subjected to influences resembling the sex hormones. Variations in the amount and nature of these hormones was indicated.

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Porphyrin Excretion of Harderian Glands in Its Relation to Actinic Carcinogenesis in Hairless Rats

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When hereditarily hypotrichotic rats and their haired litter mates were exposed to ultraviolet rays from a mercury vapor lamp, the animals of the haired series developed multiple carcinomas and sarcomas of the skin, while the animals of the hairless series reacted with an accentuation of the normal hyperkeratotic condition and the appearance of a moderate number of cutaneous horns. Only one of the hairless rats developed carcinoma of the skin (6, 7). This difference in susceptibility to actinic carcinogenesis displayed by the two types of rats was attributed to differences in the anatomical structure of their skins. The skin of the hairless rat is normally thicker and more keratotic than that of the haired rat (6, 7). Comparative studies on harderian gland fluorescence and porphyrin metabolism in cancer-susceptible and cancer-resistant animals (3) and the observation that porphyrins from the harderian glands are usually smeared on the skin of those areas that develop tumors when rats are irradiated (3) suggested the investigation of the red-fluorescent porphyrin incrustations in the two types of rats.

It was thought that if the porphyrin excretion by the harderian glands were the same in both types of rats, the porphyrin incrustations would be induced as readily and abundantly in one type of rat as in the other. This would indicate that high concentrations of porphyrins could not be one of the factors responsible for the observed differences in the susceptibility to actinic cancer. On the other hand, if a notable difference between the two types of rats with respect to the tendency to develop porphyrin incrustations could be demonstrated, support of a circumstantial nature would be lent to the contention that the disposition and metabolism of porphyrins may have an influence on light-cancer susceptibility and distribution.

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EXPERIMENTAL

Twenty-five young hairless rats (55 to 70 days old), 4 hairless rats (105 days old), and 10 haired albino rats (70 days old) were placed on a synthetic riboflavin-deficient diet to elicit porphyrin incrustations. These animals were arbitrarily designated as Group 2 of the series. The diet consisted of sucrose, 71 per cent; vitamin-free casein, 18 per cent; salts, 4 per cent; cod liver oil, 2 per cent; corn oil (mazola), 5 per cent; and the following vitamin supplements per kgm. of diet: thiamine, 5 mgm.; niacin, 100 mgm.; calcium pantothenate, 100 mgm.; *p*-aminobenzoic acid, 100 mgm.; choline chloride, 200 mgm.; and inositol, 200 mgm. The diet was given *ad libitum*. At the end of 7 weeks the surviving animals were all killed and examined in near-ultraviolet light, by means of a lamp and methods described in previous papers (1, 2). The harderian glands were removed from an equal number of haired and hairless (Group 2) rats and examined for fluorescence in ultraviolet light. The glands were then placed in acetic acid and ground in a mortar with 1 gm. of washed and ignited sea sand. The porphyrin was extracted by the method of Fischer (4). The amount of porphyrin was determined by comparative visual fluorometry, with protoporphyrin prepared by the method of Fischer and Pützer (5) from hemin derived from the red cells of human subjects as a standard.

For proper evaluation of the data thus obtained the porphyrin content of harderian glands of haired and hairless rats on an adequate diet (Group 1), and of 14 haired and 14 hairless rats irradiated (Groups 3 and 4) with a Hanovia Super "S" Alpine lamp (1 meter distance, 10 minutes daily) delivering 1,500 micro-watts at 30 inches distance from the mercury vapor burner, was determined. Seven in each group of irradiated animals were maintained on the B_2-deficient diet described previously (Group 4). At the end of the period of irradiation (4 weeks), all these animals were sacrificed for removal of the

harderian glands and extraction of the porphyrins from them.

In order to facilitate the extraction of porphyrins, the glands were ground in a mortar with 1 gm. of granulated pyrex glass. This was found to absorb only 25 μgm. of protoporphyrin as compared to a loss of 95 μgm. when sea sand was used in the earlier extraction of glands in Group 2. Each set of glands from an equal number of haired and hairless rats was extracted simultaneously and in precisely the same way. The amounts of porphyrin extracted from the sets of glands from 7 to 10 rats in each group were determined by means of a photofluorometer (Lumetron) and the amount of porphyrin per rat was calculated (see Table I).

somewhat less than in the haired rats. A reliable estimate of the differences in porphyrin content was achieved by extracting the porphyrin from the harderian glands of 10 haired and 10 hairless riboflavin-deficient rats from Group 2. Ten haired rats yielded 0.513 mgm. of porphyrin, whereas only 0.130 mgm. could be extracted from the glands of 10 hairless rats. These values include a correction for a loss of 0.095 mgm. in the sand used for grinding.

The other 6 sets of glands (Groups 1, 3, 4,) were extracted at a much later date, and yielded values that were very similar (see Table I). The harderian glands from 7 haired rats yielded 0.438 mgm. of porphyrin. These glands from the same number of hairless rats contained only 0.105 mgm. The harderian

TABLE I

Group	Diet	Treatment	Type of rat	No. of rats	Porphyrin content of harderian glands in mgm. Total	Per rat
1.	Non-deficient	None	A. Haired	7	0.438	0.063
	" "	"	B. Hairless	7	0.105	0.015
2.	B_2-deficient	None	A. Haired	10	0.513	0.051
	" "	"	B. Hairless	10	0.130	0.013
3.	Non-deficient	Irradiated	A. Haired	7	0.689	0.099
	" "	"	B. Hairless	7	0.116	0.017
4.	B_2-deficient	Irradiated	A. Haired	7	0.819	0.117
	" "	"	B. Hairless	7	0.302	0.043

RESULTS

After the rats in Group 2 had been maintained for 1 month on the riboflavin-deficient diet, 3 of the haired rats had developed distinct porphyrin incrustations of the snout and whiskers. At this time, none of the hairless rats showed porphyrin incrustations. Three days later, brown crusts on the whiskers, and other signs of porphyrin deposit, were found in all the white haired rats. Only 1 of the 16 surviving (12 young, 4 old) hairless rats showed some relatively sparse porphyrin incrustations on the nose and whiskers at this time. It was evident, therefore, that there was a striking difference between the two types of rats with regard to the tendency to develop porphyrin incrustations. These conditions remained stationary for the following 2 weeks, when 4 more of the hairless rats died. All the surviving animals were then sacrificed to permit the examination in near-ultraviolet light and to see if the harderian glands contained red-fluorescent material.

In near-ultraviolet light red-fluorescent incrusted material was seen on the noses of all 10 haired rats. A smaller amount was seen on the noses of only 2 of the 14 hairless rats examined (12 sacrificed, 2 already dead). The harderian glands from all haired rats were uniformly intensely fluorescent. The intensity of the red fluorescence of the harderian glands of the hairless rats ranged from near 0 to values

glands of haired rats maintained on an adequate or B_2-deficient diet, therefore, contained approximately 4 times as much porphyrin as those of hairless rats maintained on these same diets.

Irradiation appears to increase the porphyrin content of the harderian glands and this is most pronounced in the haired rats. The irradiated hairless rats that had been maintained on a B_2-deficient diet also yielded about twice as much porphyrin as any other group of hairless rats. For actual amounts see Table I and Fig. 1.

DISCUSSION

The harderian glands of the hairless rats contained approximately one-fourth as much porphyrin as those of the haired variety. This ratio probably reflects the porphyrin metabolism and excretion of these two sets of animals. The excretion of porphyrins by haired rats has been studied by a number of investigators (8, 9, 10), who found 0.045 to 0.1 mgm. of porphyrin excreted per rat per day. If the ratio of porphyrin in the harderian glands of the haired and hairless rats is used to calculate porphyrin excretion in the hairless rat, 0.011 to 0.25 mgm. would represent the daily excretion by a hairless rat. This is in agreement with the observation that the excreted harderian gland porphyrins caused little or no red fluorescence in the intestinal contents of the ribo-

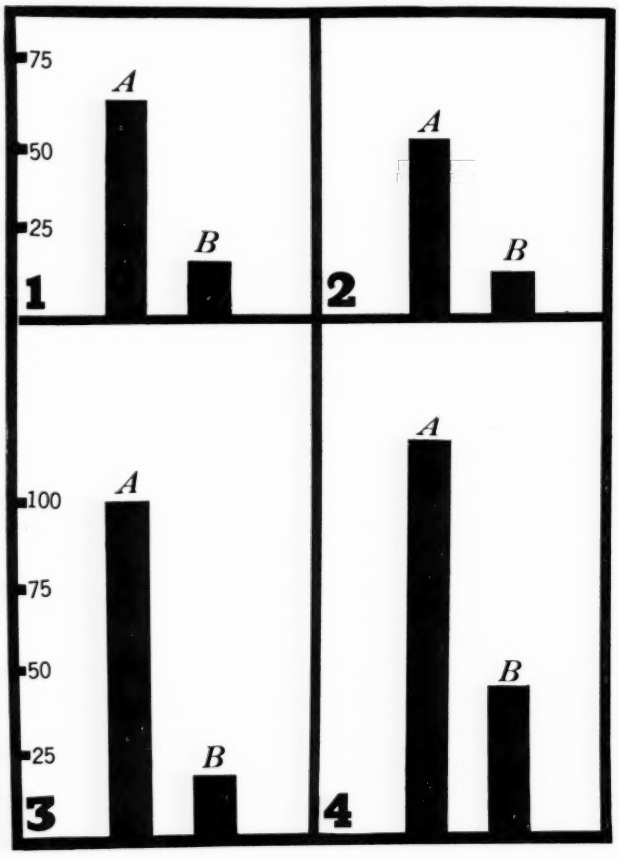

FIG. 1.—Porphyrin content of harderian glands.

A. Haired rat
1. Adequate diet
3. Adequate diet plus ultraviolet irradiation

B. Hairless rat
2. Riboflavin-deficient diet
4. Riboflavin-deficient diet, ultraviolet irradiation

flavin-deficient hairless rats, while the red fluorescence of intestinal contents and feces in the riboflavin-deficient haired rats was intense and conspicuous.

SUMMARY

1. The red-fluorescent porphyrin incrustations that may be induced so consistently in haired rats by a diet deficient in riboflavin were not induced so quickly, so consistently, or so abundantly in the hereditarily hypotrichotic rats.

2. This difference is the result of a difference in porphyrin, which is reflected in the lower porphyrin content and output of the harderian gland of the hairless rat. The harderian glands of the haired rat contain approximately 4 times as much porphyrin as the harderian glands of the hairless rat.

3. Ultraviolet irradiation of the skin increases the porphyrin content of the harderian glands in both normal haired and hereditarily hairless rats.

4. These observations may be regarded as circumstantial evidence in support of the hypothesis that porphyrin metabolism associated with the excretion of relatively large amounts of porphyrin by the harderian glands is one of the factors that influence susceptibility to light-induced cancer in rats.

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The Influence of Epidermal Cornification upon Carcinogenesis in Hairless Rats

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In studies on the action of carcinogens upon the skin normal haired animals, especially mice, which possess an epidermis distinctly different in its anatomical structure from that of man, have been mainly used. There are only three such investigations (tar, ultraviolet rays, arsenic) on record in which hairless animals were employed. Hueper (3, 4) showed that hairless rats with a thick keratotic epidermis are less sensitive to the carcinogenic action of ultraviolet rays than normal haired animals, while Lynch (6) found that hairless mice are more susceptible to tar cancer than haired mice.

During the first 2 to 3 months of life the epidermis of the hereditarily hairless rat (rhinoceros type) does not differ appreciably from that of normal haired rats, as hair follicles and sebaceous glands are properly formed. Following the progressive depilation that starts usually after the sixth week of life it exhibits, however, an increasing degree of abnormal keratinization and cellular proliferation associated with the formation of keratinized follicular cysts. Hairless rats of different age groups seemed, therefore, to represent a suitable medium for testing any possible influence that the thickness of the cornified layer and of the cellular epidermal zone may exert upon the susceptibility of the skin to carcinogenic chemicals.

EXPERIMENTAL

Methylcholanthrene (Eastman Kodak Co.) dissolved in benzol was used as the carcinogenic agent, and was applied twice weekly with a cotton swab to the lower back. Two series of hairless rats were employed in the experiment. The first, 60 in all, consisted of 10 rats 255 days old with a heavily folded, thick, loose, nodular, brownish skin; 20 rats 170 days old, of which 7 had a skin similar to that seen in the first group, while 13 had a thickened, moderately nodular, but smooth skin; 10 rats 140 days old, of which 3 had the folded and thickened skin, and 7 a thickened, but smooth skin; 10 rats 90 days old, all with a smooth, slightly thickened skin showing some scanty hair growth; and 10 rats 50 days old, all with a thin, smooth skin and a moderate hair growth. During the first months of treatment an 0.05 per cent solution of methylcholanthrene was used. The concentration was increased to 0.1 per cent during the second month and was raised again to 0.5 per cent for the remainder of the experiment.

The second series of 60 rats, which was brought into the experiment 3 months after the first one, was composed of 12 hairless rats 207 days old, 12 rats 162 days old, 12 rats 117 days old, 12 rats 97 days old, and 12 rats 77 days old. Twelve haired albino rats 120 days old were used as controls; the hair over the backs of these rats was removed at intervals with an electric clipper. Both the hairless and haired rats were painted twice weekly with an 0.5 per cent benzol solution of methylcholanthrene. The treatment of the first series was continued for 11 months, and that of the second for 8 months, when the last surviving animals were sacrificed; at this time there were 3 survivors from the first series and 4 from the second. Eleven of the 12 haired rats were alive at the end of the experimental period and were then sacrificed. For histologic study 2 pieces of skin from the painted area of the back and 1 piece from the shoulder region were used. The skin of 10 normal hairless rats ranging in age between 30 and 350 days was employed for the determination of the normal age changes.

RESULTS

The mortality among the hairless rats, especially the young ones, was high. After 3 months there were 38 survivors, after 5 months 19, and after 7 months 5 in the first series. In the second series 27 survived for 3 months and 4 for more than 6 months. Of the 7 hairless rats that thus survived to the end, 2 had been 255 days old at the start of the experiment, 2 had been 207 days old, 1 had been 170 days old, and only 2 had been 97 days old. The painted haired controls survived the entire experimental period in good health with the exception of 1 rat, which died after 5 months.

In the majority of the hairless animals the skin in the painted area became indurated, and covered by cheesy incrustations, with an occasional ulcer or abscess. In a few of the smooth-skinned animals the texture of the skin was preserved for the greater part of the experimental period, undergoing no appreci-

able changes attributable to the treatment. After 5 months of painting 4 rats showed in the painted areas small warty growths, which spontaneously receded, however, several weeks later. After 9 months a small, soft, red wart was found in 2 of the 3 surviving rats of the first series, while the third animal had a small ulcer with irregular and indurated edges. In 2 of the 4 surviving rats of the second series pinhead-sized to pea-sized papillomas were found in the dorsal region. Of these neoplastic growths only a pea-sized neoplasm with a broad base in one of the rats of the second series persisted and increased in size during the final month.

The painted areas of the haired rats did not exhibit appreciable macroscopic changes at any time; the skin remained smooth and soft, but hair growth was usually interfered with.

The skin of 40 rats of the first series and 59 of the second series of hairless rats, of 11 rats of the treated haired controls, and of 10 untreated hairless controls was used for histologic study. The painted skin of the hairless rats did not show any appreciable change during the first 2 to 3 months of treatment. During the subsequent months there developed an accentuated and exaggerated keratinization and epithelial proliferation of the epidermis as well as of the lining of the follicular sacs and cysts. The sebaceous glands were often well preserved, and seemed to be larger than those of the controls (Fig. 1); they were either attached to the outside of the follicular cysts and located at their lower pole or they formed a part of the cellular lining. While the epithelium was usually somewhat wavy in outline but sharply demarcated from the underlying or surrounding connective tissue, there occurred occasionally a minor infiltrative growth in the form of short, plump papillae or of small groups of round or oval basal cells that seemed to be dropping off into the connective tissue. The epithelial cells were regularly arranged in general, and in the superficial layers were of squamous character. Irregular arrangement was uncommon (Figs. 2 and 3). Coarse or filiform papillary projections of the epidermis covered by a thick cornified layer were found in a moderate number of instances. The filiform processes were either straight or branched and often densely placed. The epithelial lining of these projections as well as that of the adjacent epidermis consisted sometimes of elongated and spindle-shaped cells (Fig. 4). Small ulcerations of the epidermis were infrequently seen. The subepidermal connective tissue was often, but not always, edematous and moderately infiltrated with lymphocytes and monocytes. Small abscesses of the subcutaneous tissue and follicular cysts filled with leukocytes were seen on rare occasions.

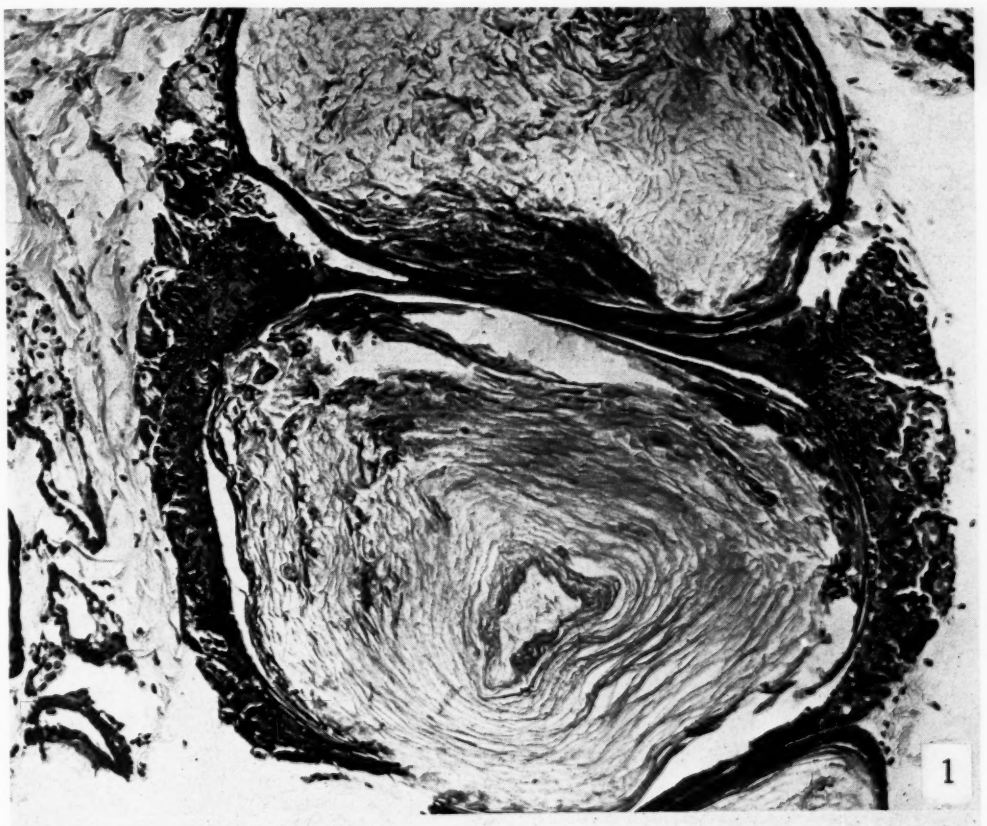
Carcinomatous lesions were found in the skin of only one hairless rat, which belonged to the second series and was 207 days old at the start of the experiment. This animal had a large pea-sized warty growth and a small ulceration with indurated edges in the painted area. The warty neoplasm was a squamous cell carcinoma exhibiting a very vascular and hyperemic stroma and imitating in places the structure of hair follicles (Fig. 5). The second tumor, which apparently originated from the lining of a follicular sac, consisted of broad and closely packed bands of large and medium-sized polygonal cells and of groups and cords of smaller, cuboidal cells with a light cytoplasm resembling those found in sebaceous glands (Fig. 6). The epithelial lining of a follicular cyst adjacent to this tumor revealed active epithelial hyperplasia which, in a restricted area, was of infiltrative type.

The sections of the painted haired rats exhibited either a normal epidermis and cutis or a mildly to moderately hyperplastic epidermis without any evidence of inflammatory reaction in the underlying connective tissue.

COMMENT AND CONCLUSIONS

The observations reported indicate that both the haired and the hairless rats possess a considerable degree of resistance to the carcinogenic action of benzol solutions of methylcholanthrene. The skin of the haired rat appears to be even more refractory than that of the hairless animal. Similar observations as to the susceptibility of haired rats to benzol solutions of methylcholanthrene have been reported by Bachmann, Cook, Dansi, de Worms, Haslewood, Hewett, and Robinson (1) and by Gordonoff and Ludwig (2). The findings on hairless rats are in agreement with those of Lynch (6) on the carcinogenic action of tar in hairless mice, and in contrast to those previously reported by Hueper (3, 4) on hairless rats exposed to ultraviolet rays. Inasmuch as the least degree of hyperplastic reaction was seen in the hairless rats with a smooth and relatively clean skin, while the most striking reactions occurred in those with a folded skin covered by cheesy incrustations, it appears as if hyperkeratinization aids in the development of cancer elicited by aromatic carcinogenic chemicals, but interferes with that caused by a carcinogenic physical agent. An abundant production of skin fat, on the other hand, seems to impair the effectiveness of both chemical and physical agents (Hueper, 5).

The observations indicate, furthermore, that the concept of cocarcinogens and anticarcinogens may have to be revised to the extent that the same agent may favor the development of cancer when present together with one carcinogenic factor, while it may hinder carcinogenesis when acting with another carcinogenic factor. It thus may appear in the role of a



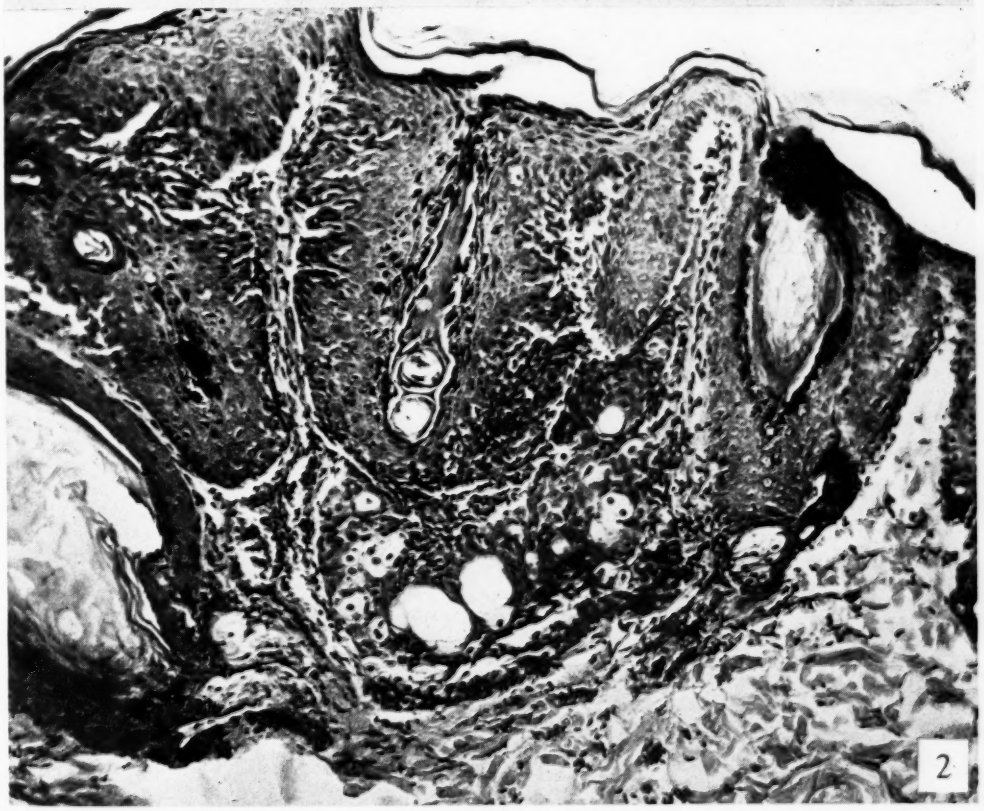
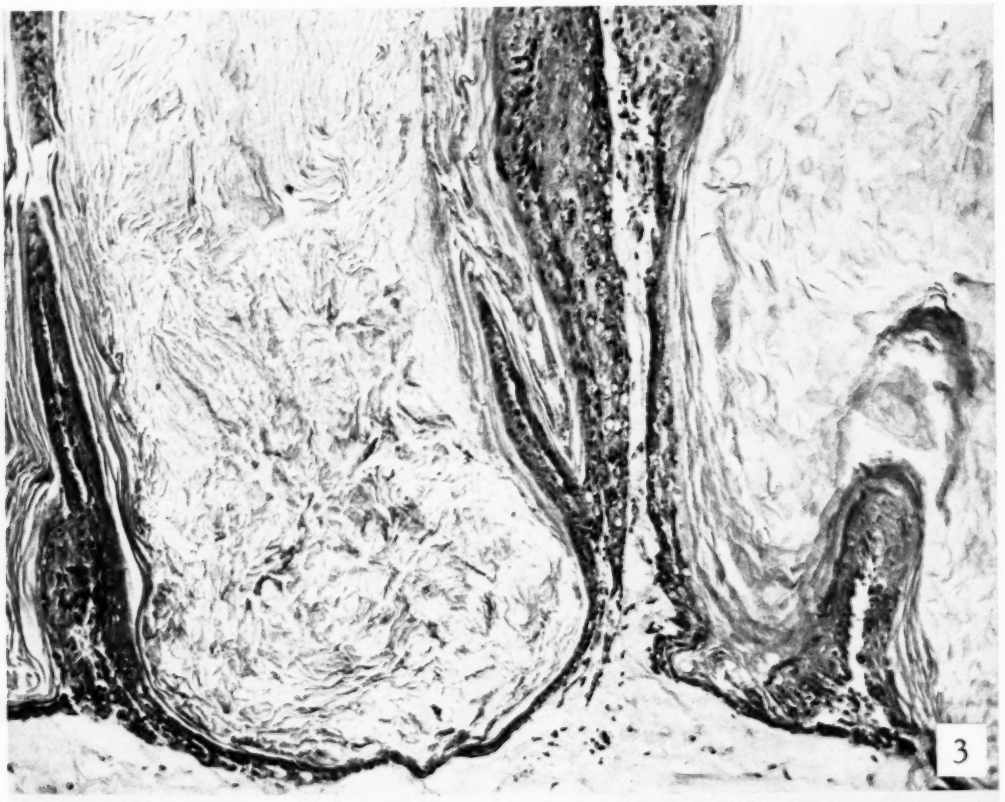


FIG. 1.—Follicular cysts with localized proliferation of epidermal lining and several groups of sebaceous cells. Mag. × 150.
FIG. 2.—Epidermal hyperplasia and hyperkeratosis showing sebaceous cells engulfed in epidermal peg. Mag. × 150.



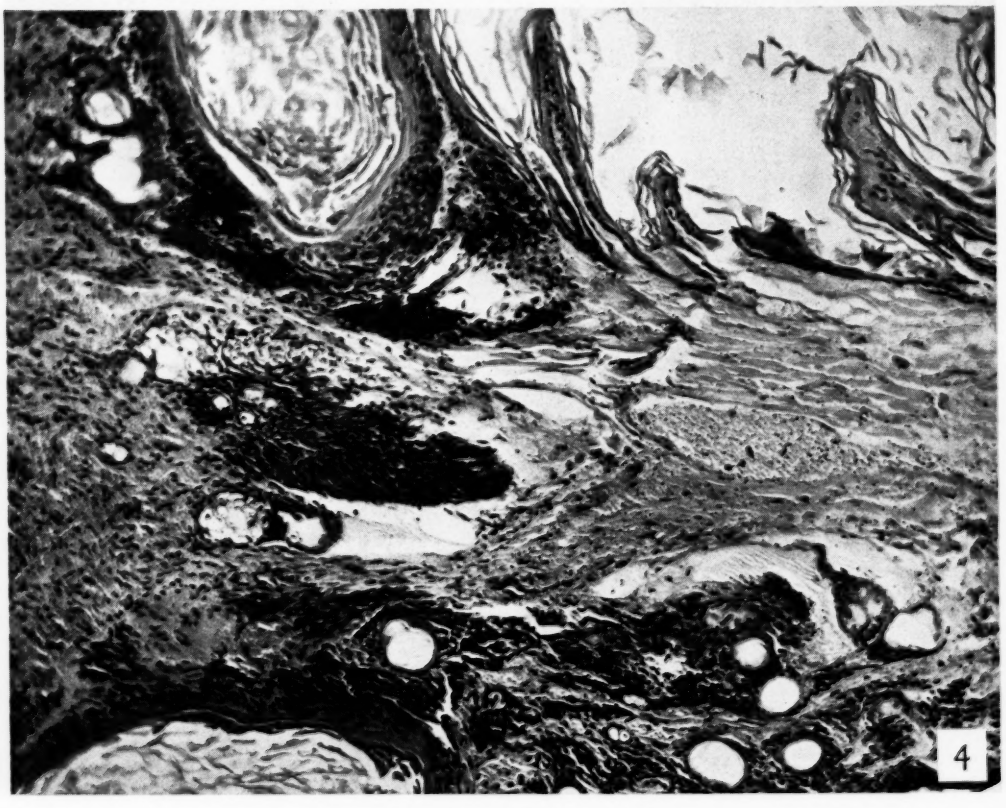
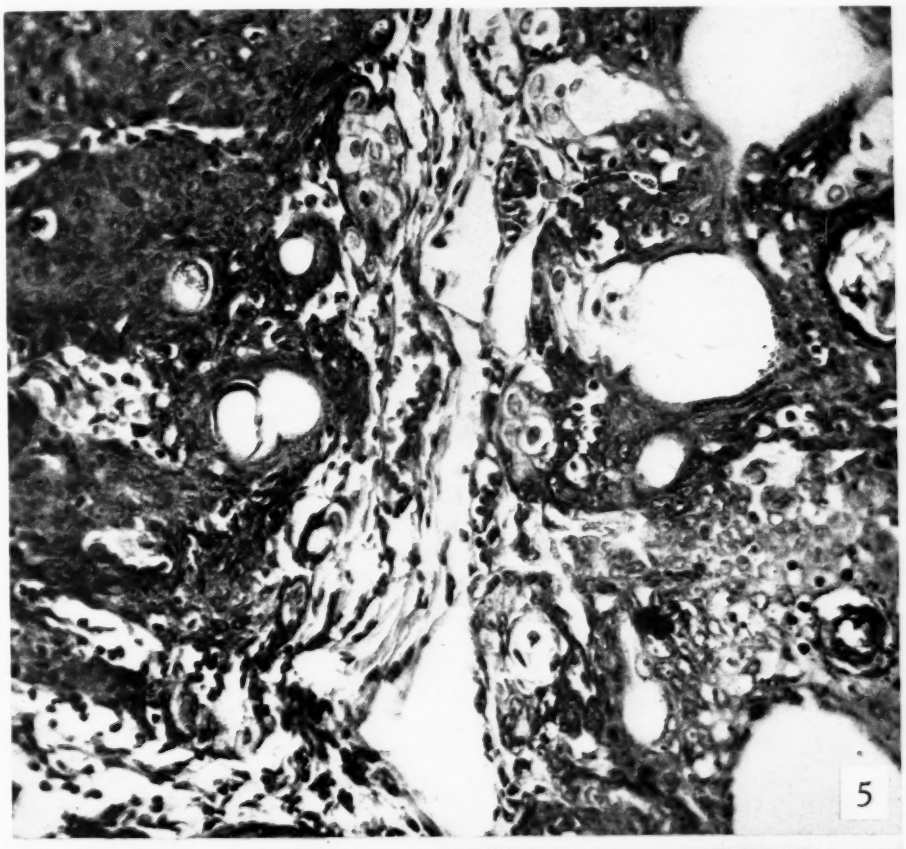


FIG. 3.—Filiform papillary hyperplasia of the epidermis with advanced hyperkeratosis. Mag. × 150.

FIG. 4.—Low filiform papillary epidermal projections with small spindle-cell proliferation in epidermis and epithelial lining of follicular cysts. Mag. × 150.



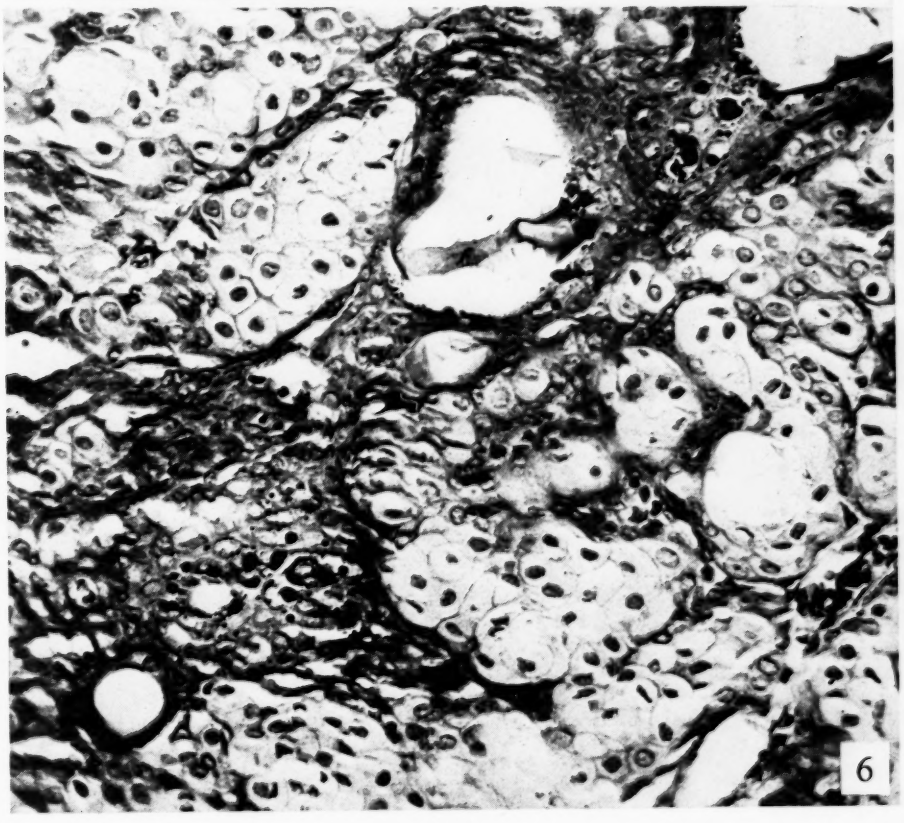


FIG. 5.—On right, round cell carcinoma with abortive hair formation and small cysts. On left, definitely hyperplastic lining of follicular cyst. Mag. × 250.

FIG. 6.—Epidermal carcinoma exhibiting large nests of clear, well outlined cells such as are seen in sebaceous glands. Mag. × 250.

cocarcinogen as well as that of an anticarcinogen, depending upon circumstances.

Hairless rats in general have a lower vitality and shorter life span than normal haired rats, and the data presented suggest that methylcholanthrene is definitely more toxic to them, particularly in early life, than to haired rats.

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The Carcinogenicity of *m'*-Methyl-*p*-Dimethylaminoazobenzene and of *p*-Monomethylaminoazobenzene*

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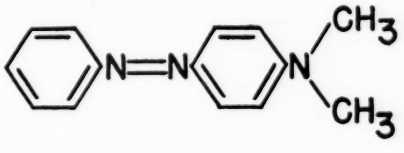
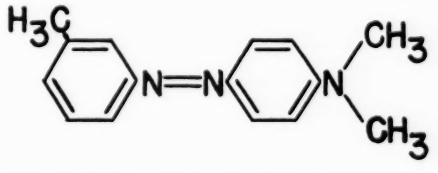
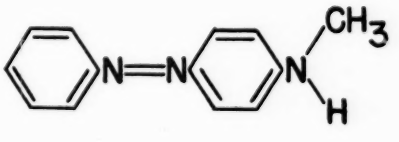
In a previous study the structure of *p*-dimethylaminoazobenzene was altered by the introduction of a methyl group into the various positions of the non-diamine ring, and it was observed that the carcinogenicity of the methylated compound depended upon the position in which the group was inserted (2). *m'*-Methyl-*p*-dimethylaminoazobenzene appeared to be more carcinogenic than *p*-dimethylaminoazobenzene. The removal of an N-methyl group from *p*-dimethylaminoazobenzene resulted in a compound, *p*-monomethylaminoazobenzene, that appeared to be at least as active as *p*-dimethylaminoazobenzene itself. The structural formulas of these azo dyes are



p-dimethylaminoazobenzene



m'-methyl-*p*-dimethylaminoazobenzene



p-monomethylaminoazobenzene

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However, the number of animals used in the initial survey was not adequate for an evaluation of the relative potencies of such active compounds. Accordingly the carcinogenicities of these three azo dyes have now been compared at several levels of administration, during several time intervals of feeding, and in two different basal rations.

METHODS

The methods employed were essentially those used previously in this laboratory (2, 3, 5). Young adult albino rats 160 to 210 gm. in weight were divided into groups of at least 12 animals and fed the rations *ad libitum* that contained the three dyes being compared. The basal diets were either a semi-synthetic one containing crude casein, 120 gm.; salts, 40 gm.; corn oil, 50 gm.; rice bran concentrate, 20 gm.; glucose, 770 gm.; and riboflavin, 0.5 mgm. per kgm.; or a synthetic diet containing vitamin-free casein (3), 120 gm.; salts, 40 gm.; corn oil, 50 gm.; glucose, 790 gm.; thiamine chloride, 3 mgm.; riboflavin, 2.0 mgm.; calcium pantothenate, 7.0 mgm.; pyridoxine hydrochloride, 2.5 mgm.; and choline chloride, 30.0 mgm. per kgm. Every rat received 1 drop of halibut liver oil monthly. Both diets have been used many times in previous studies and they are known to result in a high incidence of tumors when the carcinogen fed is *p*-dimethylaminoazobenzene.

The carcinogens were dissolved with heat in the corn oil before incorporation in the diet. Comparisons within any series were made with molar equivalents of the three dyes; *e.g.*, on a molar basis, 0.060 per cent of *p*-dimethylaminoazobenzene, the concentration used by most investigators, is equivalent to 0.064 and 0.056 per cent of *m'*-methyl-*p*-dimethylaminoazobenzene and of *p*-monomethylaminoazobenzene respectively. The exact concentrations fed and the times of feeding are indicated in Table I. At the end of the feeding period the livers were examined by laparotomy. The rats were then fed the basal diet without dye for another 2 months to permit the cirrhosis to recede, while any latent tumors originally undetected had time to develop to a recognizable size.

The *p*-dimethylaminoazobenzene was obtained commercially (Eastman No. 338). The *p*-monomethylaminoazobenzene (1) and the *m'*-methyl-*p*-dimethylaminoazobenzene were synthesized in this laboratory. The details for the preparation of the latter compound are as follows: Fifty-four grams (0.5 mole) of *m*-toluidine are dissolved in a mixture of 115 cc. of concentrated HCl and 250 cc. of water. The solution is cooled to 0° C. in an ice bath and stirred mechanically; and diazotization is effected by adding dropwise a cold solution of 34.5 gm. U.S.P. $NaNO_2$ in 150 cc. of water. The temperature of the reaction mixture should not rise above +3° C. Sixty-one grams of N-dimethylaniline and 85 gm. of anhydrous sodium acetate are then dissolved in 1,500 cc. of 70 per cent ethyl alcohol in a water bath and the solution is cooled to 20° C. The diazo solution is added all at once with stirring to the solution of the amine. The precipitate of the azo compound is filtered off and recrystallized from ethyl alcohol-water. The yield is approximately 100 gm. (Theor.=120 gm.) of recrystallized product, which melts at 119° to 120° C.

TABLE I: THE COMPARATIVE CARCINOGENICITIES OF *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE, *p*-MONOMETHYLAMINOAZOBENZENE, AND *p*-DIMETHYLAMINOAZOBENZENE IN RATS

Carcinogen	Per cent in diet	Average initial weight, gm.	Average weight increment during feeding of dye, gm.	Average daily food intake, gm./rat	Time dye was fed, mos.	Survival * at end of feeding dye	Tumor incidence †		Cirrhosis at end of feeding dye	
							At end of feeding dye	2 Months later	None-mild	Moderate-severe
A. SEMISYNTHETIC DIET										
m'-Methyl-DAB ‡	0.064	189	— 1	9.2	3	12/20	11/12	12/12	0	12
MAB ‡	0.056	190	8	9.8	3	16/20	10/16	12/16	6	10
DAB ‡	0.060	168	24	9.4	3	14/20	6/14	11/14	7	7
m'-Methyl-DAB	0.056	201	— 19	9.3	2½	13/17	0/13	13/13	1	12
MAB	0.052	190	23	11.7	2½	11/12	2/11	9/11	4	7
DAB	0.054	186	8	10.3	2½	11/12	0/11	2/11	7	4
m'-Methyl-DAB	0.048	172	18	9.5	2½	15/16	1/15	12/15	2	13
DAB	0.045	176	50	11.0	2½	15/16	1/15	8/15	13	2
m'-Methyl-DAB	0.048	202	13	10.8	2½	15/15	8/15	14/15	4	11
B. SYNTHETIC DIET										
m'-Methyl-DAB	0.064	202	14	9.4	3	8/15	7/8	8/8	0	8
MAB	0.056	195	42	12.3	3½	12/15	2/12	6/12	6	6
DAB	0.060	194	25	11.4	3½	14/15	1/14	2/14	13	1
m'-Methyl-DAB	0.048	190	59	10.6	3	13/15	3/13	8/13	8	5
DAB	0.045	193	47	11.5	3	15/15	1/15	5/15	13	2

* Survival = number living over number at start.
† Tumor incidence = number with tumors over number surviving the period during which the dye was fed.
‡ DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.

RESULTS

Large hepatic tumors developed rapidly with each of the azo dyes fed. The monomethyl compound proved to be somewhat more active than *p*-dimethylaminoazobenzene, whereas the *m'*-methyl derivative was invariably the most active of the three (Table I). For example, when 0.060 per cent of *p*-dimethylaminoazobenzene or the molar equivalent of the other compounds was fed in the semi-synthetic diet for 3 months, the mortality and degree of cirrhosis were least in the rats fed *p*-dimethylaminoazobenzene and greatest in those fed the *m'*-methyl derivative. The percentage tumor incidence at 3 months was 43 on *p*-dimethylaminoazobenzene, 62 on *p*-monomethylaminoazobenzene, and 92 on *m'*-methyl-*p*-dimethylaminoazobenzene. In the latter group the tumors were definitely larger than in those fed the other compounds.

When the concentration of the dye was reduced to nine-tenths of the original level and the feeding period shortened to 2½ months, the incidence of tumors at 4½ months in the group fed *m'*-methyl-*p*-dimethylaminoazobenzene was 100 per cent, as compared to 82 per cent for *p*-monomethylaminoazobenzene and 18 per cent for *p*-dimethylaminoazobenzene (Table I). Again the tumors were largest in the group fed *m'*-methyl-*p*-dimethylaminoazobenzene and the degree of cirrhosis was also the most severe. When *m'*-methyl-*p*-dimethylaminoazobenzene was fed for 2½ months at three-fourths of the original concentration (0.048 per cent), the incidence of tumors 2 months later was still close to 100 per cent.

Essentially the same difference between compounds appeared when a synthetic diet was used in place of

the semi-synthetic one containing the rice bran concentrate. After 3½ months of feeding the dye, the tumor incidence in the group fed *p*-dimethylaminoazobenzene was only 14 per cent 2 months later as compared to 50 per cent when the monomethyl compound was fed. In the same series, rats were fed the *m'*-methyl compound for only 3 months and the incidence of tumors reached 100 per cent shortly thereafter. When 0.045 per cent of *p*-dimethylaminoazobenzene was fed for 3 months in the synthetic diet, the incidence of tumors 2 months later, was 33 per cent, as compared to an incidence of 61 per cent when the molar equivalent of the *m'*-methyl derivative was fed.

It is thus evident that *m'*-methyl-*p*-dimethylaminoazobenzene was more carcinogenic than the other two azo dyes. At all levels of administration, at all periods of feeding, and on both basal rations, the degree of cirrhosis caused by this compound was also invariably more severe than that caused by *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene. This greater carcinogenicity of the *m'*-methyl derivative was evident in spite of the fact that the average daily food intake, and therefore the intake of dye, was less on this compound than when the other two carcinogens were fed. The high carcinogenicity of *m'*-methyl-*p*-dimethylaminoazobenzene is therefore regarded as real.

p-Monomethylaminoazobenzene also appeared to be somewhat more carcinogenic than *p*-dimethylaminoazobenzene. An extreme example of this difference was noted when nine-tenths of the molar equivalent of the original 0.060 per cent of *p*-dimethylaminoazobenzene was fed for 2½ months. The incidence of tumors 2 months later was 82 per cent when the monomethyl derivative was fed, as compared to only 18 per cent for the dimethyl compound. At other levels of administration, and on other diets, the superiority of the monomethyl derivative was less noteworthy, although it always existed. The degree of cirrhosis was also greater in the rats fed *p*-monomethylaminoazobenzene. However, the rats fed the monomethyl compound invariably ate more food, and therefore more molecules of azo dye, than those fed *p*-dimethylaminoazobenzene. Hence the question is still open whether *p*-monomethylaminoazobenzene really induces tumors more rapidly than *p*-dimethylaminoazobenzene when all other factors are adequately controlled. But that the monomethyl compound is at least as active as the dimethyl derivative is evident from the present experiments. Kensler [1] has also observed that *p*-monomethylaminoazobenzene is highly carcinogenic. Nevertheless, more data will be needed before the relative activities of the monomethyl and dimethyl dyes can be regarded as established. The question is of interest, since the two compounds appear to be interconvertible in the liver of the rat (4); roughly 3 μgm. of *p*-dimethylaminoazobenzene and 1 μgm. of *p*-monomethylaminoazobenzene are present per liver either when 0.060 per cent of the dimethyl compound is fed alone or when the molar equivalent (0.054 per cent) of the monomethyl derivative is given. Hence either compound, or the mixture of the two, or one of their metabolic derivatives could be the true carcinogen.

A somewhat higher tumor incidence was noted with *p*-dimethylaminoazobenzene when 0.045 per cent of the dye was fed than when 0.054 per cent was given in the rice bran concentrate diet; a similar situation occurred on the synthetic diet with dye levels of 0.060 and 0.045 per cent. While it is likely that the animals employed may have varied in their susceptibility to the carcinogen from series to series, it is also possible that the less extensive cirrhosis encountered on lower levels of dye allowed the tumors present to proliferate more rapidly than in the more severely damaged livers.

TABLE II: THE TOXICITY OF *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE AND OF OTHER AZO DYES IN IMMATURE RATS (SEMI-SYNTHETIC RATION)

Carcinogen	Per cent dye in diet	Initial weight, gm.	Gain in 5 weeks, gm.	Survival (5 weeks)
Control		40	39	4/4
p-Dimethylaminoazobenzene	0.054	38	12	3/4
p-Monomethylaminoazobenzene	0.052	37	14	4/4
m'-Methyl-*p*-dimethylaminoazobenzene	0.056	37	..	0/5 *
Control		92	48	3/3
o-Aminoazotoluene	0.054	95	40	3/3
p-Dimethylaminoazobenzene	0.054	89	29	4/4
p-Monomethylaminoazobenzene	0.052	93	9	4/4
m'-Methyl-*p*-dimethylaminoazobenzene	0.056	92	..	1/4

* Average survival 18 days (14 to 21 days).

Toxicity of dyes.—In addition to being the most carcinogenic of the three compounds, *m'*-methyl-*p*-dimethylaminoazobenzene was also the most toxic. This was demonstrated in a number of ways. In the prolonged experiments in which tumors were induced in adult animals, the percentage survival and the gains in weight were invariably the least in groups fed the *m'*-methyl derivative. When the dyes were fed to younger animals, *m'*-methyl-*p*-dimethylaminoazobenzene also proved to be the most toxic, as determined by the number of deaths and by the changes in weight (Table II). *p*-Monomethylaminoazobenzene appeared to be about as toxic to young rats as *p*-dimethylaminoazobenzene (Table II), but adult rats fed the mono-

[1] C. J. Kensler, personal communication.

methyl dye consumed more food and usually gained more weight than comparable animals on the other dyes. Their survival was also better (Table I). Experiments with 90 mice revealed that all three azo dyes are toxic at levels as low as the molar equivalent of 0.03 per cent *p*-dimethylaminoazobenzene, but the distribution of deaths was much more erratic in the mouse than in the rat, and no conclusions could be drawn as to the relative toxicity of the three compounds in mice.

Variation in diet.—In view of the high carcinogenicity of *m'*-methyl-*p*-dimethylaminoazobenzene, the question arose whether the formation of liver tumors with this compound was as sensitive to variations in diet as the carcinogenicity of *p*-dimethylaminoazobenzene. If so, it would be possible to save time, labor, and food by using the more active compound in dietary studies. Some sensitivity to diet was suggested by the data in Table I. When 0.048 per cent of *m'*-methyl-*p*-dimethylaminoazobenzene was incorporated in the semisynthetic diet containing the rice bran concentrate and fed for 2½ months, the incidence of tumors 2 months later was at least 80 per cent. However, when this amount of the dye was incorporated in the synthetic ration and fed for 3 months, the incidence of tumors 2 months later was only 61 per cent. Since the ration containing the rice bran concentrate definitely increases the rate at which hepatic tumors are formed when the carcinogen is *p*-dimethylaminoazobenzene (3, 5, 6), it would follow that the carcinogenicity of the more potent *m'*-methyl derivative may also be modified by diet to some extent. But a very noticeable effect of diet should not be expected in experiments in which tumors are produced so rapidly. It is recognized that modifying cocarcinogenic or anticarcinogenic influences are best demonstrated when the dosage of carcinogen employed is minimal.

SUMMARY

1. *m'*-Methyl-*p*-dimethylaminoazobenzene proved to be the most potent carcinogenic azo dye hitherto reported for the liver of the rat. On equivalent concentrations of dye rats fed the *m'*-methyl derivative invariably lost more weight, developed a more severe cirrhosis, and formed large hepatic tumors more rapidly than when *p*-dimethylaminoazobenzene was fed. When 0.048 per cent of *m'*-methyl-*p*-dimethylaminoazobenzene (¾ molar equivalent of the usual 0.060 per cent *p*-dimethylaminoazobenzene) was fed for 2½ months, the incidence of hepatic tumors 2 months later was 100 per cent.

2. *p*-Monomethylaminoazobenzene was at least as carcinogenic as *p*-dimethylaminoazobenzene. Actually more tumors developed when rations containing the monomethyl compound were fed *ad libitum* than when *p*-dimethylaminoazobenzene was fed, but the rats fed the monomethyl compound ate more food, and therefore consumed more dye.

3. In young rats *m'*-methyl-*p*-dimethylaminoazobenzene proved to be the most toxic of the three dyes.

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The Recovery of Carcinogenic Hydrocarbons from Solution by the Use of Picric Acid*

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INTRODUCTION

In a recent publication from this laboratory (2), an account has been given of the separation of neutral oils with strong blue fluorescence from the nonsaponifiable fraction of human livers. The chemical properties of these concentrates indicate that they are probably of a hydrocarbon nature, and the fluorescence suggests the possible presence of polynuclear aromatic hydrocarbons.

In the procedures that were described for the preparation of these concentrates picric acid was used. After the bulk of the sterol had been removed, the residue was dissolved in hot ethanol and the solution saturated with picric acid. It was reported that if the picric acid, which separated out on cooling the ethanolic solution, was dissolved in aqueous sodium hydroxide and extracted with ether, blue fluorescent oil could be recovered from the ethereal extract. This concentrate was freed from remaining traces of picric acid by chromatographic adsorption from hexane solution on alumina. It has since been observed that similar blue fluorescing concentrates can be obtained by high-vacuum distillation of the nonsaponifiable material after preliminary removal of most of the sterol. The concentrates obtained in this manner resemble those obtained in much poorer yields by the picric acid treatment, but do not show any reactivity towards picric acid. This observation has led us to make a detailed study of the reaction between small quantities of polynuclear aromatic hydrocarbons and picric acid. These studies have been carried out on ethanolic solutions of pure 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 20-methylcholanthrene.[1] Solutions of the hydrocarbons in ethanol containing a large excess of neutral mineral oil were examined also, in an attempt to simulate the conditions that would exist in our liver concentrates should the fluorescing components therein be of a polynuclear aromatic hydrocarbon nature.

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[1] These hydrocarbons will subsequently be referred to as "dibenzanthracene," "benzpyrene," and "methylcholanthrene" respectively.

PICRIC ACID COMPLEXES

When methanol or ethanol solutions of picric acid are added to solutions of polynuclear aromatic hydrocarbons, crystalline addition compounds frequently separate from the solution. These so-called "picrates" are, in fact, molecular complexes. They are usually much less soluble than either picric acid or the free aromatic hydrocarbon; they crystallize readily, and the color of the crystals varies from light red to black, depending on the particular hydrocarbon. They have characteristic melting points, and have been used extensively as derivatives for the purification and characterization of aromatic hydrocarbons.

If the crystalline complexes are dissolved in ethanol, they dissociate to a very considerable extent. Von Halban and Zimpelmann (4) have studied the dissociation of the picric acid complexes formed by acenaphthene and by anthracene, and recently Jones and Neuworth (3) have shown that the closely similar complexes formed between polynuclear aromatic hydrocarbons and 1,3,5-trinitrobenzene are dissociated almost completely at a concentration of 10^{-3} moles per liter in methanol. Analyses of the crystalline "picrates" indicate that they commonly contain one molecule of picric acid combined with one molecule of hydrocarbon. Weitzenböck and Klingler (5) observed that the dibenzanthracene picrate contains 2 molecules of picric acid combined with 1 of the hydrocarbon, and other cases of "dipicrate" formation may well occur.

The picric acid complex formation being an equilibrium reaction, the formation of the complex in dilute solution will be favored by a high concentration of picric acid, and in the experiments described in the following section of this paper weighed amounts of dibenzanthracene, benzpyrene, and methylcholanthrene were treated with saturated solutions of picric acid under varying conditions and the formation of the picric acid complexes studied quantitatively.

EXPERIMENTAL OBSERVATIONS

The results of our analyses are summarized in Table I. In initial experiments (I to IV), a weighed amount

of the hydrocarbon (5 to 8 mgm.) was dissolved in 25 to 60 ml. of hot ethanol. Ten grams of picric acid was added and the ethanol evaporated on an electric hot plate until a hot saturated solution was obtained. The solution was then allowed to stand at 6° C. for from 3 to 6 hours, after which the picric acid that separated was filtered off and washed with 32 ml. of cold hexane in 4 separate batches of 8 ml. each. These washings were added to the ethanol mother liquors; most of the hexane was evaporated off and the remaining solution diluted to 25 ml. with ethanol. A second treatment with 10 gm. of picric acid was carried out exactly as described above. The 2 batches of hexane-washed picric acid crystals were united, dissolved in the minimum volume of *N* sodium hydroxide solution, and extracted with 80 ml. of hexane. The picric acid passed into the alkaline phase as the sodium salt, and blue fluorescence was observed in the hexane layer. The hexane was washed thoroughly with dilute alkali and water, and dried with anhydrous sodium sulfate. On removal of the solvent on a steam bath brilliantly fluorescent crystalline residues of dibenzanthracene, benzpyrene, or methylcholanthrene were obtained.

The residues of dibenzanthracene or benzpyrene were dissolved in 10 ml. of ethanol and the concentration of the hydrocarbon was determined by ultraviolet spectrographic analysis (1). Spectrographic determination of the recovered methylcholanthrene was not possible, as the product was contaminated with yellow oily material that could not be removed by chromatographic adsorption or by washing with alkali. The amounts of methylcholanthrene recovered appeared to be smaller than those obtained from the other hydrocarbons. The dibenzanthracene recoveries ranged between 64 and 92 per cent, and the benzpyrene between 53 and 64 per cent.

TABLE I

Experiment	Hydrocarbon	Wt. of hydrocarbon taken, mgm.	Volume of ethanol solvent, ml.	Approximate wt. of picric acid used, gm.	Wt. of hydrocarbon recovered, mgm.	Recovery, per cent
I	DBA	7.50	10	10	6.90	92
II	DBA	5.40	10	10	4.58	85
	BP	8.37	10	10	4.44	53
	MCA	7.23	10	10	. . .	not analyzed
III	DBA	5.33	10	10	3.84	72
	BP	6.64	10	10	4.25	64
	MCA	7.96	10	10	. . .	not anlayzed
IV	DBA	0.93	10	10	0.59	64
V	DBA	1.54	15	6.5	1.17	76
	BP	1.55	10	4.5	0.75	48
	MCA	2.30	10	4.5	1.59	69
VI	DBA	0.294	3	1.3	0.238	81
	BP	0.211	1.5	0.75	0.042	20
	MCA	0.200	1.5	0.75	0.034	17
VII *	DBA	1.52	15	10	0.97	64
	BP	1.17	15	10	0.32	27
	MCA	1.94	15	10	0.39	20
VIII *	DBA	0.45	5	2.5	0.39	86
	BP	1.59	5	2.5	1.18	74
	MCA	0.73	5	2.5	0.26	36
IX †	DBA	5.91	10	. . . .	0.051	0.85
	BP	6.70	10	. . . .	0.14	2.1
	MCA	6.54	10	. . . .	0.25	3.9

DBA = dibenzanthracene, BP = benzpyrene, MCA = methylcholanthrene.
* Contained also 0.3 ml. of stanolax.
† Control experiment with 10 gm. benzoic acid instead of picric acid (no stanolax added).

Microscopic examination of the picric acid that crystallized from the solutions of all three of the hydrocarbons showed that two types of crystals were present; throughout the mass of plate-like, pale yellow picric acid crystals there were dispersed occasional crystals of a second kind. These were orange-colored in the material recovered from dibenzanthracene solutions, while short, dark, hair-like crystals were observed in the deposits obtained from the methylcholanthrene and benzpyrene solutions. These were undoubtedly crystals of the picrate complexes. The

crystalline deposits showed no areas of blue fluorescence such as would be indicative of the presence of crystals of the free aromatic hydrocarbons.

Attempts were made to improve the recovery of methylcholanthrene and trace the origin of the yellow by-product, which interfered with spectrographic analysis. Further purification of the picric acid did not eliminate the yellow neutral material, and it was finally observed that the formation of this contaminant could be avoided by concentrating the ethanolic solutions on a steam bath instead of an electric hot plate; presumably it resulted from a partial thermal decomposition of the methylcholanthrene.

In Experiment V, the hydrocarbons (1.5 to 2.3 mgm.) were dissolved in 10 to 15 ml. of ethanol, heated on a steam plate, and sufficient picric acid was added (4.5 to 6 gm.) to saturate the hot solution. The solutions were then allowed to stand for 24 hours at 6° C., after which the crystalline deposits were filtered off, washed 4 times with 8 ml. of cold hexane, and the picric acid was removed in the manner described above. The recoveries of dibenzanthracene, benzpyrene, and methylcholanthrene obtained under these conditions were 76, 48, and 69 per cent respectively. Microscopic examination of the picric acid deposits showed the presence of crystals of the complexes in all cases. The methylcholanthrene complex appears to separate rather slowly from solution, and in the earlier experiments (I to IV) failure to obtain appreciable recoveries of this hydrocarbon may have been a result in part of the fact that the solutions were allowed to stand for from 3 to 6 hours only before filtration. It should also be noted that where crystals of the picrate complex were observed microscopically after first treatment with picric acid, no additional recovery was obtained by a second treatment under the same conditions.

In Experiment VI an attempt was made to reduce the quantities of hydrocarbon manipulated by a factor of 10. The hydrocarbons (0.2 to 0.3 mgm.) were dissolved in 1.5 to 3.0 ml. of hot ethanol and 0.7 to 1.3 gm. of picric acid was added to saturate the hot solutions. The recoveries of benzpyrene and methylcholanthrene observed in this case were low (20 and 17 per cent respectively) but by careful control of concentrations and other conditions these could probably be raised considerably.

Recovery of Hydrocarbons in the Presence of Neutral Oils

In the experiments described above, ethanolic solutions of the pure crystalline hydrocarbons were employed. The variations in the recoveries observed with the several hydrocarbons under different conditions suggest that the controlling factor in the yield is the solubility of the picrate complex. Should small amounts of polynuclear hydrocarbons occur in neutral fat-soluble concentrates from natural sources, the solubilities of the picric acid complexes might be much greater in the presence of the oily material, and the recovery yields reduced accordingly.

In Experiment VII, the hydrocarbons (1.2 to 1.9 mgm.) were added to 0.3 ml. of "stanolax," a nonfluorescent, high-boiling liquid paraffin preparation. The mixture was heated on a steam bath with 15 ml. of ethanol, and sufficient picric acid added to saturate the hot solution (approximately 5 gm.). The solution was then allowed to stand for 24 hours at 6° C. The crystalline deposits that separated all contained scattered crystals of the darker complexes. They were washed with hexane and processed in the manner described above. From the neutral, picric acid-free fraction there were recovered 64 per cent of the added dibenzanthracene, 27 per cent of added benzpyrene, and 20 per cent of added methylcholanthrene. These residues were colorless fluorescent oils; in this respect they differed from the crystalline residues obtained in the absence of stanolax.

In Experiment VIII, 0.45 to 1.59 mgm. of the hydrocarbon was dissolved in 5 ml. of hot ethanol, 0.3 ml. of stanolax was added, and the hot solutions were saturated with picric acid and subsequently treated as in Experiment VII. In this case recoveries of 86 per cent of dibenzanthracene, 74 per cent of benzpyrene, and 36 per cent of methylcholanthrene were obtained. The residues of benzpyrene and methylcholanthrene were again noncrystalline oils; the dibenzanthracene residues contained some crystals.

In order to form some estimate of the extent to which the hydrocarbons might be carried over mechanically along with the relatively large mass of crystalline deposit, a control experiment was carried out (IX) in which 5.9 to 6.7 mgm. of the hydrocarbons was carried through a similar process, but with benzoic acid instead of picric acid; no stanolax was present. Traces of fluorescent material were recovered in all cases, but the yields were small (dibenzanthracene 0.85 per cent, methylcholanthrene, 3.9 per cent, benzpyrene 2.1 per cent.

DISCUSSION

These experiments show that the 3 commonest carcinogenic hydrocarbons may be recovered in appreciable yields from solution in ethanol by conversion to the insoluble picric acid complexes, and that this technic is adaptable to the handling of quantities of hydrocarbon of the order of 1 mgm. or less. The actual recovery yields are never quantitative and are variable, depending primarily on the concentration of the solution from which the excess of picric acid is

allowed to crystallize out. The length of time that the solutions stand before filtration is important. The recovery yields may vary greatly from one hydrocarbon to another, being much better for dibenzanthracene than for either benzpyrene or methylcholanthrene. Good recoveries of these hydrocarbons may be obtained in the presence of large excesses of neutral mineral oil, which would suggest that the technic could be of practical value for the extraction of these hydrocarbons from oily concentrates obtained from biological sources.

In the light of this additional experience, we have re-examined the behavior towards picric acid of the fluorescent concentrates obtained from the nonsaponifiable fraction of extracts of human livers (2). When the highly fluorescent neutral concentrates, obtained by distillation of the sterol-free nonsaponifiable extracts of the liver at 1×10^{-4} mm. pressure and 250 to 300° C., were carried through the procedures described in Experiments VII and VIII, no fluorescent material was recovered from the picric acid deposits. These results differ from those reported in a previous paper (2), in which less highly concentrated liver extracts were treated with picric acid and fluorescent residues recovered.

In none of the experiments with liver concentrates have we ever observed the presence of darker colored crystals on microscopic examination of the picric acid deposits, and it was failure to observe these in the earlier work that led to the suggestion that the hydrocarbon was held by a process of adsorption (2). In the experiments with added stanolax the oily, noncrystalline nature of the recovered hydrocarbons is in definite contrast with the crystalline residues obtained in the absence of such oils. This would seem to indicate that complete removal of such oils from the picrate deposit by washing is difficult to achieve. We are now of the opinion that the fluorescent material recovered from the picric acid deposits in the earlier experiments with liver concentrates was carried over mechanically and that the extraction was nonspecific; the purification (inferred from the sharpening of the ultraviolet adsorption curve) probably occurred during the subsequent chromatographic treatment that was used to ensure the removal of traces of picric acid. The fluorescent concentrates obtained by the newer high-vacuum distillation technic do not contain much oily matter, and are washed out from the picric acid deposits more easily and completely.

It does not necessarily follow from the failure to obtain evidence of picric acid-complex formation with these liver concentrates that aromatic hydrocarbons are absent; the facility with which such complexes are formed has been shown to vary very greatly from one hydrocarbon to another. At present we have no means of determining what is the actual concentration of the fluorescing substances in these extracts, and attempts at further concentration are under way.

Note added in press.—By chromatographic adsorption from hexane solution on silicic acid-celite (2:1) we have recently fractionated this fluorescent material into a number of components, which differ in their ultraviolet absorption spectra. One component is characterized by the presence of three sharp maxima at 3,320, 3,480, and 3,670 A (solvent *n*-heptane), this spectrum being identical with that of fluorescent material isolated by Zechmeister and Polgár (6) from tomatoes and from other plant sources. This substance is probably identical with, or similar to, "iso-anhydro vitamin A." A second fluorescent component of our liver concentrate has a single sharp maximum near 2,850 A.

SUMMARY

It has been shown that small quantities of dibenzanthracene, benzpyrene, and methylcholanthrene of the order of 0.5 to 5 mgm. may be removed from ethanolic solution by conversion to picric acid complexes. These complexes separate from the solution together with excess picric acid when a hot ethanolic solution of the hydrocarbon is saturated with picric acid, cooled to 6° C., and allowed to stand for 24 hours. The excess picric acid may easily be removed and the free hydrocarbon recovered from the picric acid complex. The procedure has been studied quantitatively, the yields of recovered hydrocarbon being estimated by ultraviolet spectrophotometry.

The recovery yields vary greatly with the particular carcinogenic hydrocarbon, being of the order of 70 to 90 per cent for dibenzanthracene, 30 to 60 per cent for benzpyrene, and 20 to 40 per cent for methylcholanthrene. Satisfactory recoveries have been obtained in the presence of large excesses of "stanolax," a neutral mineral oil; from this it may be inferred that the technic is applicable to the extraction of small amounts of carcinogenic hydrocarbons from neutral oils of biological origin.

Fluorescent neutral oils can be obtained from the nonsaponifiable fractions of human livers after removal of sterol by crystallization, chromatographic adsorption, and high-vacuum distillation. These concentrates have been treated with picric acid under conditions similar to those that have been shown to lead to the extraction of carcinogenic hydrocarbons from mixtures with added stanolax, but no evidence of the formation of picrate complexes has been observed in these cases. These results differ from those reported by one of us (R. N. J.) in an earlier paper, in which experiments were described wherein cruder liver extracts were treated with picric acid and some evidence suggestive of a preferential extraction of fluorescent material by the picric acid was observed. A possible cause of these different results is discussed.

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Influence of Unsaturated Dibasic Acids on the Induction of Skin Tumors by Chemical Carcinogens*

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The process of carcinogenesis may be retarded or accelerated in many ways. Apart from the isolation, by controlled breeding, of strains of mice of varying susceptibility to special forms of cancer, the use of short-wave radiations, a wide range of chemical compounds, hormonal imbalance, and dietary supplements or deficiencies have all achieved some measure of success. It is difficult to envisage a single and specific mechanism influenced in a common way by such a diversity of artificial technics. Though it is premature to generalize, a consideration of the known properties of several of the chemical agents used supports the view that a disturbance of sulphur metabolism may play a determining role in their biological action.

A study of the effect of substances that interfere with specific metabolic processes and the induction rate of skin cancer by chemical carcinogens has supported this conception. Many hydrolyzing halogen compounds possess inhibitory powers, and the potency of their anticarcinogenic action runs parallel with their chemical reactivity (7). Though many biochemical processes, including that of glycolysis, could be affected by compounds of this type, the possibility that they operate primarily as disturbers of sulphur metabolism is supported by the finding that bromobenzene, with a nonreactive halogen atom, but known to be removed from tissues largely as a sulphur derivative, possesses a similar anticarcinogenic action (8). No doubt other substances normally detoxicated through mercapturate formation would exhibit the same property. But the thesis that sulphur is involved in the early stages of skin carcinogenesis would be better established by the use of substances that are structurally unrelated to the types mentioned above, and that lead to the fixation of sulphur by a different chemical mechanism.

This paper records the use of simple dibasic unsaturated acids as inhibitors of carcinogenic action, and presents evidence that they function by interference with S metabolism.

* Because of the difficulties of international communication the author has not read proof of this article.

EXPERIMENTAL

Inhibitory Action of Maleic Anhydride on the Induction of Skin Tumors by 3,4-Benzpyrene

The mixed strains of mice used, the dietary regimen, and the technic of applying the carcinogen have been described in a previous communication (8). Maleic anhydride was used in preference to maleic acid on account of its somewhat greater solubility in fat solvents; its action, owing to its rapid hydrolysis in aqueous media, is the same as that of the acid itself. Dissolved in ether plus 2 per cent of medicinal liquid paraffin in suitable concentrations, it was applied widely over the benzpyrene-treated areas 4 times weekly on the days between the benzpyrene treatments. A rough estimate shows that about 1 mgm. of maleic anhydride covered the benzpyrene-treated area when the maximum concentration of 6 per cent was used. If the skin remained free from gross lesions due to other causes, no visible skin damage other than permanent freedom from hair was noticeable, and the mice retained their normal weight and good health throughout the longest experimental periods.

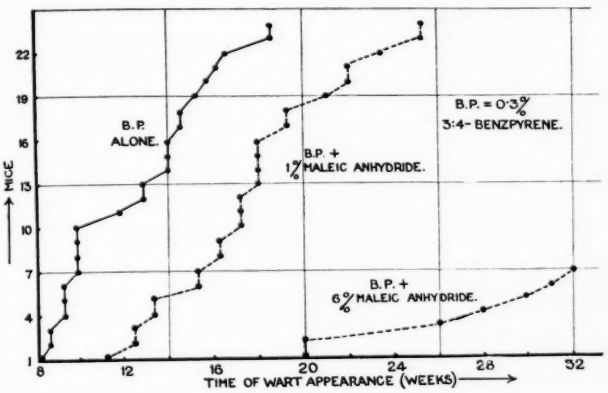
Twenty-five mice were used for each experiment and representative results are pictured in Fig. 1. They show that the inhibition of carcinogenic action increased with the concentration of maleic anhydride applied. When this attained a certain level, exemplified by 0.3 per cent benzpyrene plus 6 per cent maleic anhydride (Fig. 1A) or 0.1 per cent benzpyrene plus 4 per cent maleic anhydride (Fig. 1B), it was possible to delay the advent of tumors for a period during which all the surviving mice in control groups carried papillomas or epitheliomas. The progressive loss of hair, a characteristic sequel to interference with S metabolism, in itself suggests the nature of the action of maleic anhydride, but further evidence that its inhibitory action is due to fixation of SH-containing cell constituents will be given later.

Inhibitory Action of Maleic Anhydride on Tumor Induction by 1,2,5,6-Dibenzanthracene

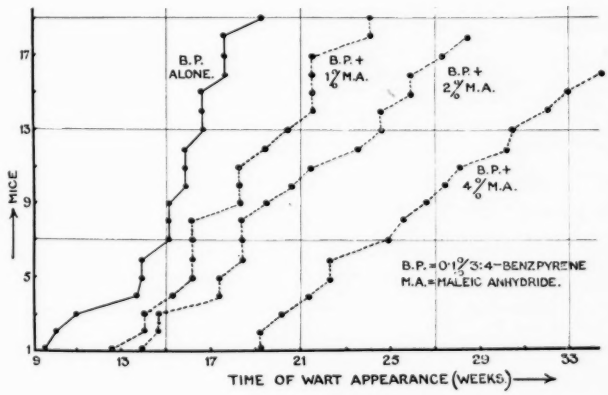
The inhibition of tumor induction by maleic anhydride is not unique for the carcinogen 3,4-benzpyrene.

Similar effects were produced when 1,2,5,6-dibenzanthracene, the only other carcinogen available, was used.

The technic of application was similar to that described above, except that 0.2 per cent dibenzanthracene in acetone plus 2 per cent medicinal paraffin was substituted for benzpyrene. All treatment was discontinued after 9 months, and the experiment was ended after 12 months. A typical result is summarized in Table I.



A. 0.3 per cent 3,4-benzpyrene.



B. 0.1 per cent 3,4-benzpyrene.

FIG. 1.—Retarding effect of maleic anhydride on the rate of tumor induction by 3,4-benzpyrene (25 mice in each group).

Though combination of maleic anhydride and dibenzanthracene can take place under appropriate conditions (5), it is unlikely that this reaction plays any role in the observed inhibition, since the addition compound appears to dissociate into its components after injection into mice.

Effect of Citraconic Acid, Malonic Acid, and Aldehydes on the Rate of Tumor Induction by 3,4-Benzpyrene

It has long been known that addition reactions can occur between mercaptans and unsaturated compounds. They are most successful when α,β-unsaturated carbonyl compounds are used, though addition of mercaptans to unsaturated hydrocarbons is not uncommon. Combination is normally effected in the presence of HCl, sodium ethylate, piperidine, etc., but interaction in some cases occurs under physiological conditions of temperature and pH. Morgan and Friedmann (11) showed that maleic acid combines with thioglycollic acid, cysteine, and glutathione in buffer solutions at pH 7.4 and 37° C. They examined the kinetics of these reactions and isolated the addition compounds. Under their conditions (maximum concentration $= M/12.5$) no interaction was observed when other acids related to maleic, *e.g.*, citraconic, mesaconic, cinnamic, etc., were used. On the basis of these results citraconic acid would seem to be a suitable control to maleic acid in the demonstration that the latter's power of retarding tumor development is indeed due to its combining capacity for sulphydryl groups.

Animal experiments similar to those described above were carried out, in which citraconic anhydride was substituted for maleic anhydride. They showed (Fig. 2) that citraconic acid is equally effective as an inhibitor. A reinvestigation of the possible reaction of citraconic acid with cysteine and glutathione was therefore undertaken. It was found that combination does take place at higher concentrations to an extent determined by the relative masses of the reactants. The experiments were conducted in Thunberg tubes at 37° C. and pH 7.4 under strictly anaerobic conditions. Cysteine hydrochloride was exactly neutralized with $2N$ NaOH, the sodium salt of the unsaturated acid added, and the mixture brought to the desired molarity by the addition of Sørensen's phospate buffer. Alternate evacuation and flushing with hydrogen were repeated several times, for spurious results were easily obtained without this precaution. Controls that measured the autoxidation of the SH compounds, particularly when cysteine was used, were essential. Glutathione was added directly to the sodium salt of the acid dissolved in phosphate buffer. After incubation for suitable periods the residual SH compound was estimated by titration with $N/100$ iodine, and a correction made for autoxidation. Table II includes a selection of the results obtained.

At the higher concentrations used in these experiments addition between maleic acid and SH compound approached completion in 30 minutes. Though citraconic acid added much more slowly than this and the maximum combination attained during 5 hours was about 60 per cent, the biological potencies of the two acids were substantially the same. Further reference will be made to this finding in the section dealing with skin-glutathione determinations. Yet it is clear that the results shown in Fig. 3 can be reasonably attributed to the specific disturbances of S metabolism caused by citraconic acid.

No further investigation of the possible anticarcinogenic action of other α,β-unsaturated carbonyl compounds has been undertaken as yet. But, by way of contrast, other simple substances with known chemical and biochemical properties have been used as additional controls. Two of these, furfuraldehyde and malonic acid, gave results that are illustrated in Fig. 2. In each case a 4 per cent solution was applied by the standard technic already described. They provide a demonstration that the intermittent treatment of mouse skin with chemically reactive substances at fairly high concentration level is inadequate, in itself, to cause substantial inhibition of carcinogenesis. Yet they must produce disturbances of a physicochemical nature that are temporary and nonspecific.

Furfuraldehyde gave results representative of several aldehydes (mainly of the aliphatic series containing up to 8 C atoms) that have been tested. Schubert (14) showed that reaction between aldehydes and SH-containing compounds can be achieved in aqueous solution under mild conditions approximating those found physiologically. The degree of combination and the nature of the product varied with the particular aldehyde and SH compound chosen, simple addition leading to semimercaptals, or, where molecular conditions were favorable, to the formation of a thiazolidine ring by further condensation. The products all exhibited a high degree of dissociability, varying with the aldehyde component used and the alkalinity of the medium. These chemical characters were reflected when aldehydes were used in experiments with carcinogens. Even though some fixation of SH groups might well occur in the cells, the easy dissociation of the formed compounds would permit only fleeting disturbances of S metabolism, which could be correlated with their small inhibitory effect on tumor induction.

Malonic acid, though highly reactive, does not combine directly with the SH group. It has been used in biochemical studies as a specific inhibitor of succinic dehydrogenase (10, 13). It functions as a competitive substrate, and its effectiveness depends on the ratio of its concentration to that of succinic acid. The result of an experiment in which it was used in conjunction with 3,4-benzpyrene is shown in Fig. 2. No significant delay occurred in the rate of tumor development. This negative result of a specific interference with another phase of metabolism emphasizes, by contrast, the special role of S metabolism in the carcinogenic process.

In previous unreported experiments an attempt had been made to influence the course of tumor induction by maintaining a state of lowered respiration at the site of application of a carcinogen. The compound used was α-naphthoyl cyanide, which slowly hydrolyzes under physiological conditions and liberates

TABLE I

	No. of mice used	No. of mice in which tumors developed	Time of appearance of first wart, weeks	No. of survivors without tumors after 12 months	Average induction time of tumors, weeks
0.2% dibenzanthracene alone	30	24	13.5	0	19.5
0.2% dibenzanthracene + 4% maleic anhydride	30	12	24.0	14	35.5

TABLE II: COMBINATION OF CITRACONIC ACID WITH SH COMPOUNDS AT 37° C. AND pH 7.4

Concentration of cysteine, *M*	Concentration of citraconic acid, *M*	Reaction time, hours	Combination, %
0.8	0.2	4	11
"	0.6	4	18
"	1.3	4	29
"	3.0	4	42
"	9.0	4	70
0.4	2.0	1	18
"	"	3	29
"	"	5	40
0.4	*M*	1	7
"	"	3	15
"	"	5	24
0.1	2.0	4	10
"	6.0	4	29

Concentration of glutathione, *M*	Concentration of citraconic acid, *M*	Reaction time, hours	Combination, %
0.3	0.5	4	14
"	1.3	4	25
"	2.6	4	37
"	9.0	4	57
0.3	2.5	1	18
"	"	3	41
"	"	5	55
0.1	2.5	1	5
"	"	3	17
"	"	5	26
0.03	2.5	1	4
"	"	3	9
"	"	5	12

HCN. No demonstrable effect on tumor induction occurred, though probably the desired conditions were never attained because of rapid dispersal of the HCN in the general circulation.

Effect of Unsaturated Dibasic Acids and Other Substances on the Glutathione Content of Mouse Skin

Changes in the S metabolism of mouse skin induced by treatment with maleic, citraconic, and malonic acids and several aldehydes were studied. The substances were applied in 5 per cent ethereal solution to the backs of mice epilated 2 days previously. Four applications were given at half-hourly intervals, and the chemical estimations were made at chosen times after the final painting. A total of about 20 mgm. of the reagent was therefore spread over the back of each mouse. This represented an amount roughly equal to that applied during 1 week in the experiments with carcinogens, and greatly accentuated the metabolic conditions prevailing throughout those experiments. The skins of untreated mice were found to be remarkably uniform in their content of glutathione, with deviations from the average rarely exceeding 10 per cent.

The whole skins of the backs of 5 mice were used for each estimation. Details of the technic employed have been given previously (8). The ascorbic acid content of the skin was found by titration with phenol-indo-2,6-dichloro-phenol and its iodine equivalent deducted from the value of total reducing substances titratable with iodine. As was found in the case of bromobenzene any fall in the glutathione skin content was accompanied by a proportionate fall in the ascorbic acid level. The points on the curves shown in Fig. 3 represent the value for glutathione after this correction had been made. Though experiments *in vitro* showed that the velocity of the reaction between maleic acid and SH-containing compounds is several times greater than the corresponding reaction with citraconic acid, the action of these two acids on S metabolism in the skin was very similar both in speed and in intensity. The time of recovery to normal glutathione and ascorbic acid levels was substantially the same in both cases.

The curves indicate that the action of these acids when used in conjunction with carcinogens was operative only for 4 short periods of a few hours during any one week. It is arresting that such comparatively slight intermittent interferences with S metabolism can be responsible for the pronounced retardation of the carcinogenic process shown in Figs. 1 and 2.

When the skins of mice were similarly treated with malonic acid or any of the lower aliphatic aldehydes no effect on S metabolism was detectable. If the conception that S metabolism is involved in the early stages of carcinogenesis be correct, this finding would be in agreement with their innocuous behavior towards tumor induction.

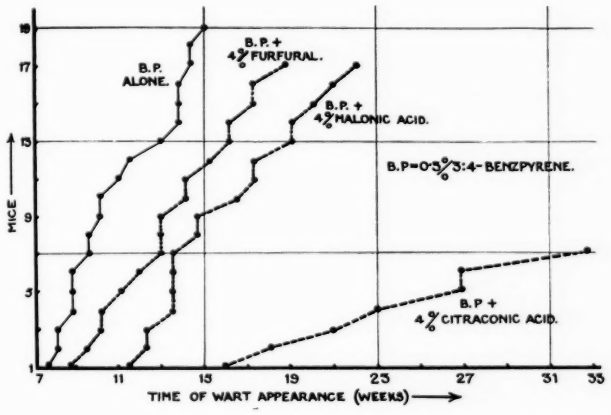


Fig. 2.—Effect of citraconic acid, malonic acid, and furfural on the rate of tumor induction by 3,4-benzpyrene (25 mice in each group).

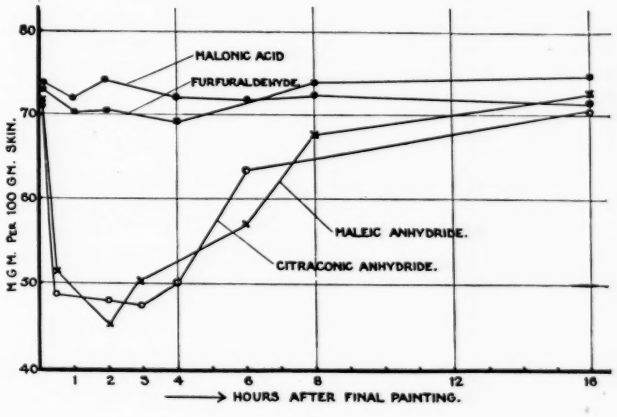


Fig. 3.—Changes in the glutathione content of mouse skin treated with maleic anhydride, citraconic anhydride, malonic acid, or furfuraldehyde.

Effect of Maleic Anhydride on a Precancerous Area of Skin

Many experiments have been carried out in which a limited treatment with carcinogen has been followed by application of an inhibitor of S metabolism. All, in varying degree, show that the emergence of tumors from potentially cancerous skin is delayed or prevented by this procedure.

The best conditions for realizing these effects are those in which the treatment with carcinogen is stopped after warts have emerged in 1 or 2 mice; at this point interference with S metabolism is begun. More extended carcinogenic action, by increasing the rate of wart formation, makes the demonstration of inhibitory effects less easy, while shorter action unduly prolongs the appearance of warts in the control mice. A sample experiment is illustrated in Fig. 4.

In this case, 0.3 per cent 3,4-benzpyrene was applied to 2 batches each of 30 mice twice weekly for 8 weeks. At this time 1 wart was visible in each batch. The control group was then left untouched and papillomas emerged in 14 mice over the next 28 weeks. Each mouse in the other group received one No. 6 brushful of 4 per cent maleic anhydride spread widely over the precancerous area 4 times weekly. After 28 weeks papillomas had been observed in 5 mice; 2 of them had meanwhile regressed. Twenty mice of this group remained tumor-free and in good condition throughout the whole experimental period.

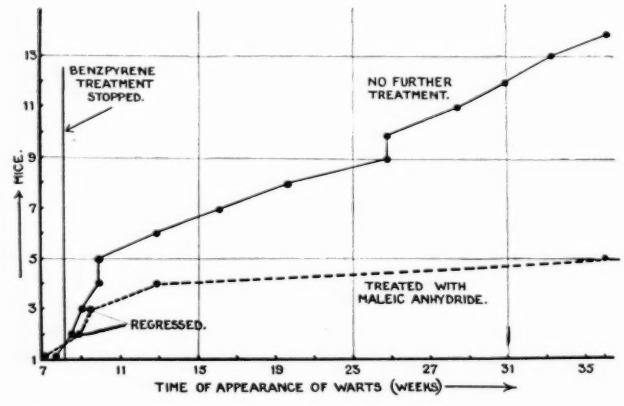


FIG. 4.—Delay and prevention of tumor emergence in a precancerous area of skin treated with maleic anhydride (30 mice in each group).

OBSERVATIONS ON THESE RESULTS

Three classes of chemical substances have been shown to delay or prevent the completion of chemical carcinogenesis in mouse skin. They are:

(a) Hydrolyzing halogen compounds (7). (b) Compounds that are eliminated by mercapturate formation (8). (c) α,β-Unsaturated dicarboxylic acids.

The factor common to the chemical and biochemical activities of these 3 types of inhibitors is the power of lowering the level of S metabolism through combination with SH-containing cell constituents by the 3 different chemical processes of condensation, oxidative coupling, and addition respectively.

By contrast, a limited number of other classes of substances, which are known to interfere specifically with other phases of cell metabolism, have been shown to have little or no effect on the carcinogenic process. The data are inadequate to permit wide generalization, but the conception that S metabolism plays a role in the primary action of chemical carcinogens has emerged and can be further explored.

The possibility that the substances used operate solely as growth inhibitors by depriving the tissues of essential S amino acids has been previously discussed (8). It appears inadequate to account for the results shown in Fig. 4. In this type of experiment the carcinogen has initiated processes that, within limits, can be reversed, and this reversibility is conditioned by the intermittent fixation of SH groups within the cell. When treatment with inhibitor is stopped after 26 weeks, and conditions for normal growth are restored, there is no recurrence of carcinogenic action, though several tumors emerge in the controls after this period. A temporary inhibition of growth has been accompanied by a permanent reversal of the carcinogenic process. The simplest hypothesis that would account for these facts is that a co-operation of carcinogen and SH-containing cell constituents is an essential first stage of carcinogenic action.

Present knowledge would suggest that the carcinogen, unchanged or slightly modified, had entirely disappeared long before tumors emerged in the controls of the experiment illustrated in Fig. 4. (9); *i.e.,* that the chemical stimulus of a biological process was inoperative for several months before its characteristic action became manifest. If the carcinogen were the sole cause of neoplastic change, as is widely conceived, this paradoxical phenomenon would defy easy interpretation.

The alternative view that some residue of the applied carcinogen, probably in altered form, still lingers within the cell during the long latent period is equally tenable, though lacking proof. On this view the action of inhibitors of S metabolism in reversing the carcinogenic process would be rationally explained. They would be influencing a continuing process, not a completed one. Also, it would in no way conflict with the known facts relating to the detoxication of carcinogens, which are eliminated mainly as oxidized products lacking carcinogenic activity and with no known association with the mechanism of malignant change (1, 4).

The interfering action of the 3 classes of substance mentioned above demonstrates that S metabolism is directly concerned in the process that culminates in new growth. An unequivocal statement on the part played by sulphur in the elimination of carcinogens cannot yet be made. Though no S-containing detoxication products have so far been isolated, the work of White (15, 16) can most easily be interpreted on the assumption that sulphur is involved either directly or indirectly in the metabolism of carcinogens both of the hydrocarbon and azo types.

Were this the case it would be possible to envisage two reactions running concurrently:

(a) Detoxication of carcinogens by processes involving sulphur. (b) Combination of carcinogens, or their derivatives, through S linkages as a primary phase of the carcinogenic process.

The presence of an inhibitor of S metabolism would retard the detoxication reaction through competitive

action, and tend to lessen the rate of removal of the carcinogen as a S derivative. If S were concerned *only* in this elimination process, more prolonged carcinogenic action would therefore be possible under these conditions of S depletion, and an increase in the rate of tumor induction would be anticipated. The experiments described negative this possibility.

But, if reaction (b) occurred, then an inhibitor of S metabolism, by curtailing this process, would cause a retardation, or even a reversal, of carcinogenic activity. The experiments are in harmony with this conception.

The working hypothesis emerging from all these experiments is that a primary stage in the mode of action of chemical carcinogens is their fixation, through free SH groups, to cell constituents, and thus an alteration of the biochemical potentialities of cell enzymes. At this stage no further speculation will be offered.

Potter (12) has made a tentative approach to the possible role of SH-containing enzymes in the carcinogenic action of *p*-dimethylaminoazobenzene on the liver. The products of metabolism of azo dyes are readily accessible for enzyme studies *in vitro,* but their power of depressing the functional activity of enzymic SH groups does not clearly indicate the mechanisms involved in carcinogenesis. Less is known of the action of derivatives of carcinogenic hydrocarbons on special enzyme systems. Alkali-soluble products of unknown constitution, obtained by the photochemical oxidation of some of these hydrocarbons were shown by Boyland (2, 3) to inhibit the action of lactic and succinic dehydrogenases and to depress the total respiration and glycolysis of a number of normal and tumor tissues. Yet these derivatives no longer behaved as carcinogens, and thus conformed to the findings of Wood and Fieser (17) that modification of the carcinogenic hydrocarbons by introduction of chemically active functional groups leads to a loss of biological potency.

SUMMARY AND CONCLUSIONS

1. The carcinogenic action of 3,4-benzpyrene and 1,2,5,6-dibenzanthracene on mouse skin is greatly retarded by maleic and citraconic anhydrides. The same substances delay and often prevent the emergence of tumors in a precancerous area of skin.

2. Malonic acid, α-naphthoyl cyanide, and several (mainly lower aliphatic) aldehydes failed to influence the carcinogenic process.

3. Evidence is given from experiments *in vitro* and *in vivo* that the unsaturated dibasic acids combine with SH-containing cell constituents.

4. It is inferred that the inhibition of carcinogenesis is specifically correlated with the intermittent lowering of S metabolism, and that interference with other phases of metabolism has little influence on carcinogenic action.

5. The hypothesis that a first stage in the action of chemical carcinogens is their fixation, through S linkages, to cellular enzymes, is discussed.

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Milk-Induced Mammary Carcinoma in Mice*†

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Bittner's discovery (4) in 1936 that the development of mammary carcinoma in mice is dependent, in part at least, upon a factor present in the mothers' milk has been confirmed in several laboratories. Andervont and his associates (1-3, 7) at the National Cancer Institute, in particular, have added considerably to our knowledge of the phenomenon. But so much remains unknown that we think it worth while to make a report of our own progress with the study of the milk factor. Our experiments were begun in 1940, and are still continuing. Since this kind of experiment is complete only after the experimental mice have died of old age, progress is slow.

We had at our disposal for the study 2 well controlled strains of mice that we had bred by brother and sister matings in our laboratory over a period of years. These are the RIII and the C57 black. We have described in detail elsewhere (6) the environment of our mouse colony. Briefly, the mice are kept 1 pair in each cage, allowed to breed without interference, and are fed a standard diet of pellets manufactured by the Arcady Farms Milling Company, of Chicago. Under these conditions our mice reach an advanced age.

Foster Nursing

In carrying out the foster-nursing experiment, we made a special effort to transfer the newborn mice from their own mothers to their foster mothers as soon after birth as we could, so that the young mice would get as little of their own mothers' milk as possible. The cages were inspected morning and evening and newborn mice transferred at these times. Yet those mice that were born during the night were with their own mothers for as long as 16 hours, and no doubt got some of their milk.

The foster-nursed mice, on reaching maturity, were paired off brother and sister and bred in the usual way. Their offspring, the F_1 generation in the foster-nursing experiment, were allowed to nurse from their own mothers and were reared and bred in our customary manner. We did not carry the experiment beyond the F_1 generation, since we are primarily interested in identifying the unknown factor present in milk, and not in tracing the influence of this factor through subsequent generations of mice.

When mammary tumors developed, the mice were sacrificed and a routine autopsy was done that included taking sections of the breasts, adrenals, thyroid, cervix, uterus, and ovaries. The mice that did not develop tumors were kept caged in pairs until they died of natural causes, when they, too, were carefully autopsied.

Our data, arranged according to a statistical method that we have used before, follow. The controls were in part the mothers of the mice that were foster nursed. A control series running concurrently with the experimental series of animals is thus provided. We believe that in experiments of this type, carried out with so-called "pure" strains of mice, it is important to provide a new control series for each experiment. Even though these strains have been bred by brother and sister matings for many years they are not biologically pure, and changes in the disease incidence of the strain continue to occur.

In the interpretation of these results it is interesting first of all to note that the incidence of mammary carcinoma in the bred female controls of our RIII strain has risen considerably during the last few years. In our last published control series (6) the incidence was 73.57 per cent, while it has reached 93.9 per cent in the present series. This is probably due to the selection of breeding stock from the offspring of tumor-bearing mothers, which although not an invariable rule is certainly our tendency.

The C57 controls here reported had no tumors. But when C57 young were nursed by RIII mothers 76.1 per cent of them developed carcinoma. One of these mice bearing multiple mammary carcinomas is shown in Fig. 1. This is the highest incidence of mammary carcinoma induced in the C57 strain by foster nursing that has been reported. In his most recent paper, Bittner, for instance, says that he obtained carcinoma in only 18.4 per cent of his C57 mice when they were foster nursed by his high carcinoma A strain (5).

* Work completed before Major Randall joined the A.U.S.

† This work was aided by a grant from the Leggett and Watters Cancer Research Fund.

TABLE I: CONTROLS—BRED FEMALES

	RIII strain	C57 strain
Total group: mice surviving 6 months	195	150
Percentage in which mammary carcinoma developed	92.9 ± 1.84	0
Mean age in months at death	12.6 ± .32	23.5 ± .22
S.D. of age in months at death	4.45 ± .23	2.68 ± .16
Median age in months at death	11.8 ± .40	24.0 ± .27
Mice in which carcinoma developed	181	0
Mean age in months at death	11.9 ± .26	
S.D. of age in months at death	3.46 ± .18	
Median age in months at death	11.3 ± .32	
Mice in which carcinoma failed to develop	14	150
Mean age in months at death	21.5 ± 1.30	23.5 ± .22
S.D. of age in months at death	4.87 ± .92	2.68 ± .16
Median age in months at death	24.0 ± 1.62	24.0 ± .27

See footnotes to Table III.

TABLE II: FOSTER-NURSED BRED FEMALES

	RIII strain	C57 strain
Total group: mice surviving 6 months	83	92
Percentage in which mammary carcinoma developed	53.0 ± 5.48	76.1 ± 4.34
Mean age in months at death	15.4 ± .58	17.6 ± .5
S.D. of age in months at death	5.32 ± .41	4.83 ± .36
Median age in months at death	14.7 ± .73	16.2 ± .63
Mice in which carcinoma developed	44	70
Mean age in months at death	12.3 ± .52	15.8 ± .39
S.D. of age in months at death	3.43 ± .37	3.26 ± .28
Median age in months at death	12.05 ± .65	15.5 ± .49
Mice in which carcinoma failed to develop	39	22
Mean age in months at death	18.9 ± .75	23.2 ± 1.07
S.D. of age in months at death	4.69 ± .53	5.03 ± .76
Median age in months at death	18.7 ± .94	24.0 ± 1.34

See footnotes to Table III.

TABLE III: F_1 GENERATION FROM FOSTER-NURSED BRED FEMALES

	RIII strain	C57 strain
Total group: mice surviving 6 months	100	100
Percentage in which mammary carcinoma developed *	63.0 ± 4.83	69.0 ± 4.63
Mean age in months at death †	15.7 ± .57	16.5 ± .53
S.D. of age in months at death ‡	5.72 ± .4	5.32 ± .38
Median age in months at death §	14.9 ± .72	14.95 ± .67
Mice in which carcinoma developed	63	69
Mean age in months at death	12.5 ± .48	13.7 ± .44
S.D. of age in months at death	3.78 ± .34	3.67 ± .31
Median age in months at death	12.0 ± .6	13.3 ± .55
Mice in which carcinoma failed to develop	37	31
Mean age in months at death	21.2 ± .68	22.5 ± .54
S.D. of age in months at death	4.12 ± .48	2.98 ± .38
Median age in months at death	23.0 ± .85	23.2 ± .67

* Standard error for percentage $= \sqrt{\frac{P \times Q}{N}}$. P = percentage positive, Q = 100 − P, and N = number of cases.

† Standard error of mean $= \frac{S.D.}{\sqrt{N}}$

Standard error of standard deviation $= \frac{S.D.}{\sqrt{2N}}$

‡ Standard deviation (S.D.) $= \sqrt{\frac{\Sigma d^2 F}{N} - (bx)^2}$

§ Standard error of median = 1.25 standard error of mean.

The high incidence that we have obtained is strong evidence for the predominance of the nursing factor in the etiology of mammary carcinoma in mice.

The mean age at which the foster-nursed C57 mice developed carcinoma was 15.8 months, which is about 3 months later than the disease appears in the RIII strain. This relatively late appearance of the disease in the C57 mice suggests that the agent in the milk is not the only factor concerned in the induction of the disease. Its action appears to be modified by constitutional factors in the strain.

FIG. 1.—C57 mouse No. 1677, foster nursed by an RIII strain mouse. Multiple mammary carcinomas at the age of 13.2 months.

The reduction of the incidence of the disease in the RIII mice foster nursed by C57 mothers was not so striking, the decrease being only from 93.9 to 53 per cent. There was no change in the mean age at which these RIII foster-nursed mice developed their carcinomas.

In the F_1 offspring of these foster-nursed mice these changes in carcinoma incidence and in the age at which the tumors developed persisted. They were, however, somewhat less notable, which suggests that although the influence of the milk factor is transmitted to some degree through successive generations it must be renewed through ingestion of milk itself for each generation of mice if it is to exert its full effect.

ARTIFICIAL FEEDING

In an attempt to exclude the possibility of the transference from mother to offspring of some agent other than milk that might play a part in the development of carcinoma, we carried out a series of experiments in which young C57 mice were removed from their mothers and fed milk artificially obtained from RIII mothers. We used an electrical milking machine modelled after one designed by Andervont and his associates. The process of obtaining milk in this way is rather tedious, but with care about 1.5 cc. can be obtained from a mother that is nursing a good sized litter. The eighth to the tenth day of lactation are the best. Only one milking of each mouse is possible, for enough trauma is done to the nipples to discourage the young from further nursing, and lactation ends.

Feeding the newly born mice artificially is a difficult feat. In our hands dropper feeding worked best. When removed from their mother as soon after birth as possible the young mice were kept in an incubator and at hourly intervals during the day were taken out for feeding. A small medicine dropper was used and a small drop of milk placed on the tip of the tongue of the mouse. When this was swallowed the process was repeated until 3 or 4 drops had been taken. It was found that the mice could be nourished in this manner during the day, but if we continued the artificial feeding during the night they died. The best that we could do was to feed them by dropper from 9 o'clock in the morning until midnight, and then return them to their own mothers during the night, keeping up this routine for 3 days. At the end of this time the mice were returned permanently to their C57 mothers. The mice were thus largely nourished upon C57 milk, receiving only a small quantity of RIII milk during their first 3 days of life. Our results with this method are shown in Table IV.

It is difficult to calculate with any degree of exactness how much RIII milk each mouse actually received by this method; certainly it was not more than 1 cc. In several of the mice the amount was considerably less. One mouse, C57 No. 3557, for example, was removed from its mother at birth and dropper-fed with RIII milk only during the afternoons of the 2 following days, after which it was returned to its C57 mother. It could scarcely have received more than 0.2 cc. of RIII milk. Yet this was sufficient to induce the development of a mammary carcinoma at the age of 18.6 months. It is interesting to speculate upon the nature of an agent which, introduced into an animal

in such a minute amount during the first 2 days of life, causes the development of a malignant neoplasm in old age. The action of viruses, as we now know them, is not so long delayed.

TABLE IV: C57 MICE FED ARTIFICIALLY WITH RIII MOUSE MILK

Total group: mice surviving 6 months	68	
Percentage in which mammary carcinoma developed	42.6	± 5.99
Mean age in months at death	20.9	± .57
S.D. of age in months at death	4.74	± .41
Median age in months at death	19.65	± .72
Mice in which carcinoma developed	29	
Mean age in months at death	15.7	± .53
S.D. of age in months at death	2.84	± .37
Median age in months at death	15.80	± .66
Mice in which carcinoma failed to develop	39	
Mean age in months at death	24.5	± .54
S.D. of age in months at death	3.35	± .38
Median age in months at death	24.2	± .67

SUMMARY

1. Foster nursing has produced mammary carcinoma in 76 per cent of the females of a strain of mice in which there were no mammary carcinomas among the control animals.

2. A method of artificial feeding has shown that as little as 0.2 cc. of milk are sufficient to induce mammary carcinoma.

3. The carcinomas thus induced appear at a considerably later period in the life of the mouse than is usual in high mammary carcinoma strains.

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Studies on the Variation of the Rous Sarcoma Virus Following Growth of the Tumor in the Anterior Chamber of the Guinea Pig Eye*

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Mammalian cancer from a wide variety of tissues and from a diversity of species has been successfully transplanted into the anterior chamber of the eyes of rabbits and guinea pigs (4, 5). The growth of benign tumors, or of potentially malignant neoplasms during their preinvasive stages, invariably fails to occur in this environment, and it has been suggested that the property of heterotransplantability is an important characteristic of cancer. It was of interest, therefore, to see if the Rous sarcoma, a typical representative of the infectious avian tumors, could be grown in the anterior chamber of a mammalian eye, and further, if growth occurred, to compare the tumor's behavior with that of morphologically comparable growths of unknown etiology. Moreover, inasmuch as Duran-Reynals (2, 3) has shown that growth of the Rous sarcoma in different avian species results in a modification of the properties of the virus, it was considered important to check the tissue and species specificities of the Rous agent following passage of the tumor through the anterior chamber environment.

METHODS

The Rous sarcoma used in this study was obtained from a strain that has been carried in the laboratory of Dr. Duran-Reynals for the past 6 years. It has been kept in desiccated form, with passage through Plymouth Rock chickens every 6 or 8 months and subsequent desiccation of the developing tumors. The sarcoma from this source is referred to in the present paper as "stock Rous."

Guinea pigs 4 to 6 months of age and of either sex were used as mammalian hosts for the avian tumor. Transplantation into this rodent was effected by opening the anterior chamber of the eye at the sclerocorneal junction with a double-edged keratome knife; a fragment (2 to 3 cu. mm.) of tumor was then inserted by means of a trocar and forced toward the lower portion of the chamber by applying slight pressure to the corneal surface. In all cases except one, the tumors utilized for transfer were obtained from chickens freshly killed. In one case the tumor was taken from a bird found dead.

Ten to 12 days after transfer the guinea pigs were killed, the tumor was removed from the eye under sterile precautions, and inoculated by trocar into the breast muscle of Plymouth Rock chicks varying in age from a week to 10 days. In a few cases the transplants were returned to the anterior chambers of other guinea pig eyes in an attempt to pass the tumor serially in this species. Tumors obtained in the chicken following growth in the guinea pig eyes will be referred to in this paper as "Rous ACT."

Tumors developing in the chickens inoculated with growths from guinea pigs were transferred to other chickens either by the injection of 1 ml. of cell suspension (dilution 1/5 by weight with normal saline) into the breast muscle of normal birds, or by the injection of Berkefeld "N" filtrates (dilution 1/20 by weight with normal saline), 1 ml. intravenously plus 1 ml. intramuscularly in the breast. Cultures for detecting bacterial contamination were taken on all suspensions and filtrates. For the routine passage of the ACT strains Plymouth Rock chicks from 1 to 2 weeks old were used. In 1 instance Peking ducks were inoculated during the first 24 hours of life with Rous ACT, and attempts were made to establish a Rous ACT line in this species.

In a few instances the Rous tumor was passed alternately from chicken to guinea pig to chicken and back to guinea pig.

Serial passages of stock Rous tumor were used as controls in these experiments. Fragments of the tumor used for guinea pig inoculation were injected directly into chicks and subsequent passages into other chicks,

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** This work was done while the senior author was a Fellow of The International Cancer Research Foundation.

1 to 2 weeks old, were made by cell suspensions and sometimes by Berkefeld "N" filtrates.

Both control and experimental birds were kept together in the same brooders, but in cages separate from other animals infected with different virus-induced tumors.

RESULTS

The Growth of the Rous Sarcoma in the Anterior Chamber of the Guinea Pig Eye

The Rous sarcoma was transferred in 5 different experiments from chickens to the eyes of 38 guinea pigs. It was observed in the first place that the majority of sarcomas grew, provided that the tumor was obtained from a living bird and was not contaminated with bacteria. While growth occurred in the one instance in which the tumor was obtained from a dead chicken, it was felt that material from this source was inferior to that derived from recently killed animals. Secondly, it was noted that, in general, vascularization of the transplant occurred very soon after transfer, a matter of 3 or 4 days. Growth occurred rapidly at first, the diameter of the tumor doubling or trebling during the first 2 weeks. Thereafter, however, the tissue remained quiescent and showed no change in size for as long as 6 months. During this period the tumor appeared alive, for on microscopic examination no degenerative changes were seen and occasional mitotic figures were found.

The behavior of morphologically comparable mammalian cancers in heterologous species differs from that of the Rous sarcoma in several particulars. As a rule the appearance of vascularization is delayed for a week or more, and no increase in size may be observed for several months. The tumors then grow rapidly to fill the chamber (5, 6).

Another point of dissimilarity lies in the difficulty with which the Rous sarcoma was passed serially in the guinea pig. In the case of mammalian tumors serial transfer is easily effected and apparently may be continued indefinitely, but to date the Rous sarcoma has been successfully passed for only 2 consecutive transfers in the guinea pig. On the other hand, after a single passage in the chicken, return to the guinea pig is followed by growth. However, subsequent transfer back again into chickens from the second guinea pig has not as yet been successful. This latter observation will be a subject for further study.

The histology of the Rous sarcoma remained fairly constant regardless of the environment in which it was placed or the history of its passages. Figs. 1 and 2 show the tumor as it appears in the chick. In this species the morphology may vary from a rather compact fibroblastic tumor to one in which the fibroblasts are widely separated. In Figs. 3 and 4 it is evident that the cellular character of the tumor has not changed as a result of its residence in the anterior chamber of the guinea pig eye. In like manner the microscopic picture was not changed significantly by reinoculation into the chicken (Figs. 5 and 6), or a second serial passage in the guinea pig (Fig. 7). There was no evidence of increased collagen content of the tumor after guinea pig passage as described in the case of the duck-adapted Rous virus (2). Chicks inoculated with the ACT strains developed tumors at distant sites in a manner similar to those injected with stock Rous (Figs. 8 and 9).

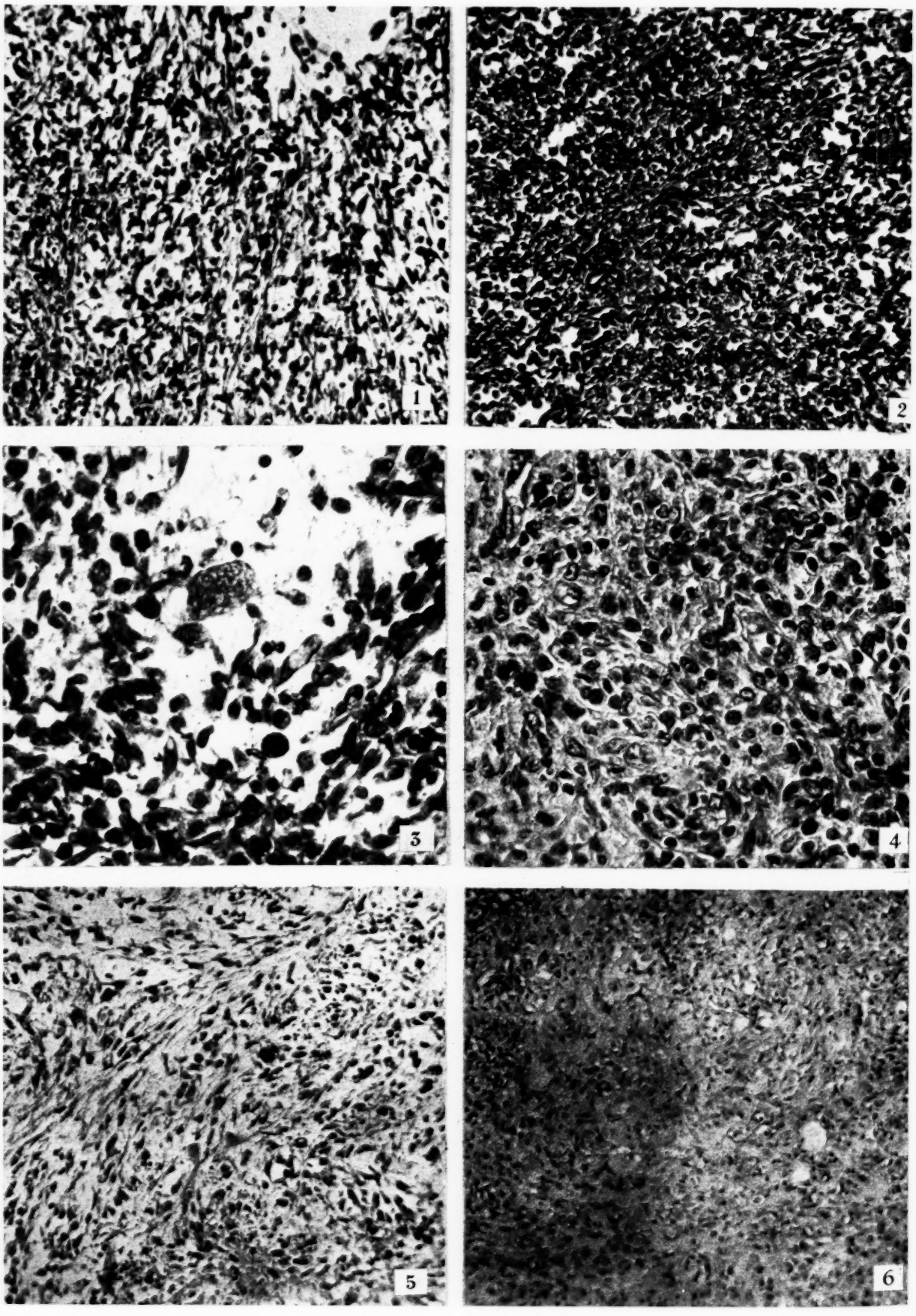
In one experiment the anterior chamber of the eye was irrigated with a very dilute cell suspension of the stock Rous tumor, prepared by grinding the tissue in saline and centrifuging. The irrigation was accomplished by means of openings in both the upper and lower portions of the anterior chamber. A 27 gauge needle attached to a 1 ml. syringe was inserted into the upper opening and about 0.5 ml. of the suspension was injected; the overflow fluid was allowed to escape through the lower hole. Within 3 days after this procedure tumor growth was evident on the border of the iris. Microscopic examination revealed numerous actively dividing cells embedded in a moderate amount of hyalinized material (Fig. 10). Attempts were also made to infect the guinea pig by irrigating the anterior chamber with an active Berkefeld "N" filtrate of the Rous tumor in a manner similar to that described above. However, all the results so far have been negative, as shown by inoculation of the cornea and chamber contents into chicks.

To date there has been no evidence that the Rous sarcoma growing in the anterior chamber of the eyes of guinea pigs spreads to other parts of the host either by metastasis or by extension. The effect of the Rous virus on the guinea pig is a subject now under study.

The Variation of the Rous Virus Following Growth of the Tumor in the Anterior Chamber of Guinea Pig Eyes

Growths of the Rous sarcoma from the guinea pig eye were transferred to chickens in 4 of the 5 experiments referred to above, and in 3 separate instances it was possible to establish lines of the Rous ACT tumors in chickens. In the fourth experiment 7 of 8 birds inoculated from guinea pigs showed tumors by palpation from 6 to 11 days after injection, but by the 28th day all the growths had regressed. These birds were killed on the 96th day after inoculation and no evidence of the growth was present.

In the other 3 experiments tumors from guinea pigs occurred at the inoculation site in 11 of 15 birds in from 2 to 16 days after injection, the average being 10 days.



FIGS. 1-6

Most of the tumors in the first chicken passage were firm, fairly well localized, and ranged from 1 to 2 cm. in diameter at the time the animals were killed. In addition hemorrhagic disease, which is a nonneoplastic manifestation of the tumor virus (1), was present in 2 of the birds, showing that the actual virus had been carried through the pig transfer. No periosteal tumors were observed in any of the birds inoculated directly from the pigs. Because the transplants from the anterior chambers were small, it was impossible to make filtrates of any of the growths from this source.

Three strains of tumors were established from the 11 chickens into which Rous ACT growths were passed and these were carried for 6, 7, and 8 passages respectively. For convenience each tumor strain will be discussed separately, since there is evidence to suggest that the Rous virus did not vary in a similar manner after each guinea pig passage.

Rous ACT I.—This strain was carried for 8 passages involving 165 birds. Both cell suspensions and Berkefeld "N" filtrates were used in making transfers. The fact that the virus had been modified by guinea pig passage first became evident when filtrates were employed in inoculation. Birds so injected developed periosteal tumors of one or more of the long bones (Fig. 11). These tumors histologically (Fig. 12) showed a proliferation of the periosteal cells and actual invasion of the compact bone resulting in bone destruction. Endosteal tumors were also found in some cases, but it was uncertain whether the component fibroblasts were derived from the endosteum or whether the cells from the periosteal tumor had penetrated the cortex of the bone at some undetected spot and had proliferated within the bone marrow. The formation of new bone following primary destruction, as has been described in chicks inoculated with the duck variant of the Rous virus (2), was not observed.

The incidence of bone tumors in each passage of ACT I is shown in Table I. The occurrence of such lesions, as may be seen, is greatly dependent upon whether passages were made with cell suspensions or filtrates, and by far the greater percentage followed the use of the latter. Only birds that lived 8 days or longer after inoculation and were within the age range of from 1 day to 3 weeks at the time of injection are included in the table.

In many ways the behavior of Rous ACT I in chicks was similar to that of stock Rous (1). Hemorrhagic disease of the liver and spleen was observed frequently in the ACT I strain, and there was a decrease in the extent of blebs and correspondingly a greater abundance of tumors as chicks of increasing age were inoculated. Hemorrhagic blebs were found with varying regularity in the gastrointestinal tract, pancreas, gonads, kidney, muscle, and bone marrow. While hemorrhagic lesions in these localities have been relatively rare in the stock Rous, as previously observed (1), they were not seen at all in the controls of the present experiment. Hemorrhagic blebs in the lung were very common with this particular strain of ACT. The highest incidence (40 per cent) occurred in the second passage, while in subsequent transfers the frequencies dropped to approximately 10 per cent.

Rous ACT II.—One hundred and forty chickens distributed over 6 passages were involved in the maintenance and study of this strain. Again, the most conspicuous characteristic of Rous ACT II, like ACT I, was the production of periosteal tumors. Chart I shows the distribution of these and other lesions among a portion of the birds used in the study of the ACT II tumor. Table II indicates the relative frequency of bone tumors following the injection of filtrates. In the sixth passage one bird inoculated with cell suspension of the ACT II tumor developed periosteal tumors in 3 of its long bones.

ACT II, unlike ACT I, caused very few cases of hemorrhagic disease involving the lungs, only 8 birds being so affected. It is interesting to note that the incidence of successful infection of chicks by this virus, as manifested by sarcomas or hemorrhagic disease, was less with this strain than with the ACT I. Forty-four per cent of the chicks inoculated with ACT II virus failed to show lesions, in contrast to 17 per cent failure in the ACT I strain. The filtrates were tested by inoculation into susceptible chicks, and were found to produce tumors in all cases with the single exception of a batch used in one series of 6 animals.

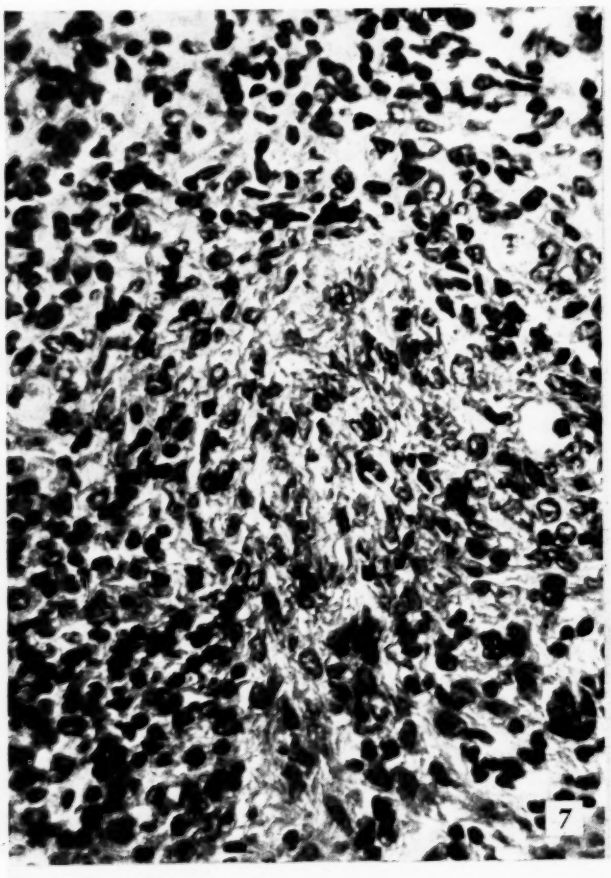
Rous ACT III.—Many birds inoculated with this strain of the Rous variant showed bone lesions. However, such tumors did not become evident until the third chicken passage, when filtrates were used. In this passage 4 of 9 birds showed bone lesions. In the fourth passage none of the 5 animals receiving filtrate developed periosteal growths, but 1 bird inoculated with cell suspension bore tumors in 3 of its long bones. Six of 14 birds in the fifth passage, and 3 of 16 in the

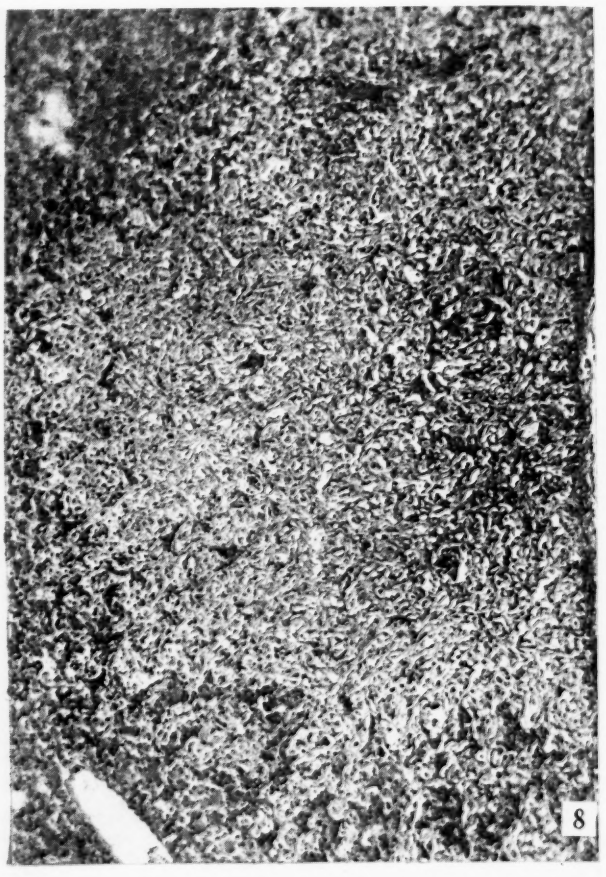
DESCRIPTION OF FIGS. 1 TO 6

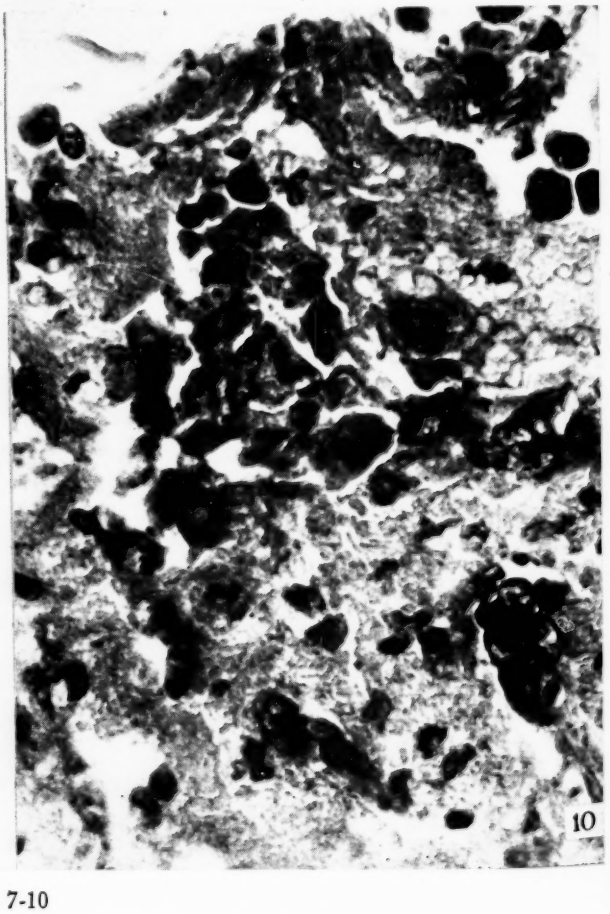
FIGS. 1 and 2.—Variation in architecture of stock Rous sarcoma in chickens. Mag. × 250.

FIGS. 3 and 4.—Rous sarcoma growing in guinea pig eye. Mag. × 450.

FIGS. 5 and 6.—Rous sarcoma in chicks following growth in guinea pig eye. Note architectural types and compare with Figs. 1 and 2. Mag. × 250.







FIGS. 7-10

sixth transfer, possessed bone lesions. In the final passage (seventh) 1 chick showed a periosteal tumor.

The pronounced tendency toward hemorrhagic disease of the lungs noted with ACT I was not apparent with this strain, although 11 of the birds did show such lesions. Moreover this strain appeared to retain much of its species specificity, for in contrast to the observation made with ACT II virus only 3 of the 49 birds (6 per cent) inoculated with filtrate failed to show clinical manifestations of the virus infection.

Rous ACT III inoculated into ducks.—In an attempt to determine whether or not Rous ACT III would grow in ducks, 24 hour old Peking ducks were inoculated intramuscularly with cell suspensions made from tumors of the second chicken passage. Takes occurred, and it was possible to maintain the resulting tumors for 5 consecutive passages by the use of cell suspensions. While filtrates of these tumors failed to produce growths in ducks, a filtrate of the tumor in its fourth duck passage produced typical hemorrhagic disease in chicks. Likewise, the injection into 2 week old chicks of cell suspensions of the duck tumor from its second passage resulted in hemorrhagic disease in the liver and the development of periosteal tumors.

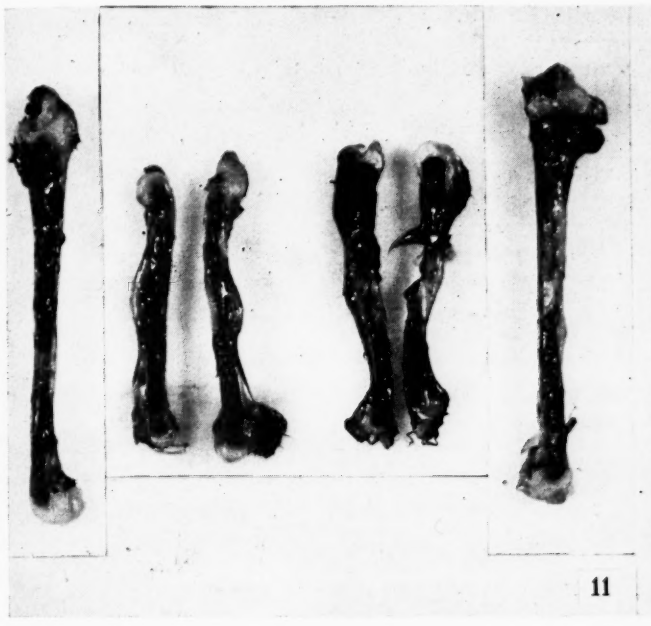


FIG. 11.—Bone lesions in a bird inoculated with Berkefeld "N" filtrate.

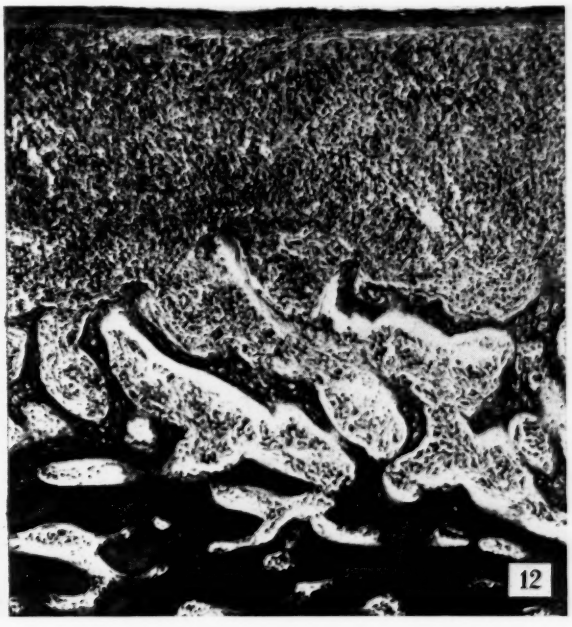


FIG. 12.—Bone lesion showing proliferation of periosteal cells and compact bone destruction. Mag. × 100.

TABLE I: PERCENTAGE OF BIRDS SHOWING BONE TUMORS IN EACH PASSAGE OF ACT I

Passages	Inoculated with filtrates		Inoculated with cell suspension		Total no. inoculated	Percentage of total no. with bone tumors
	No. of birds	Percentage with bone tumors	No. of birds	Percentage with bone tumors		
1	0	0.0	4	0.0	4	0.0
2	5	80.0	17	5.9	22	22.7
3	8	25.0	22	4.5	30	13.3
4	10	50.0	32	0.0	42	11.9
5	0	0.0	24	4.2	24	4.2
6	0	0.0	12	8.5	13	8.5
7	5	60.0	4	0.0	9	33.3
8	7	28.5	2	0.0	9	22.2

DESCRIPTION OF FIGS. 7 TO 10

FIG. 7.—Rous sarcoma in second guinea pig passage. Mag. × 450.

FIGS. 8 and 9.—Lesions of Rous ACT III distant from inoculation sites, in liver and lung respectively. Mag. × 100.

FIG. 10.—Growth of Rous sarcoma in anterior chamber resulting from irrigation of eye with tumor extract. Mag. × 700.

Table III shows the total number of ducks used in each passage and the number showing tumors. Unfortunately all the birds in the sixth passage died before sufficient time had elapsed for the development of sarcomas, and as a result the ultimate fate of the ACT III tumor in ducks is not known.

ACT transfers. In one case the stock Rous was carried for 6 passages involving 146 birds. In no instance was hemorrhagic disease of the lungs seen, nor were hemorrhagic lesions of the gastrointestinal tract, pancreas, or kidney noted. Hemorrhagic blebs were found in the bone marrow on one occasion. Four birds of 38

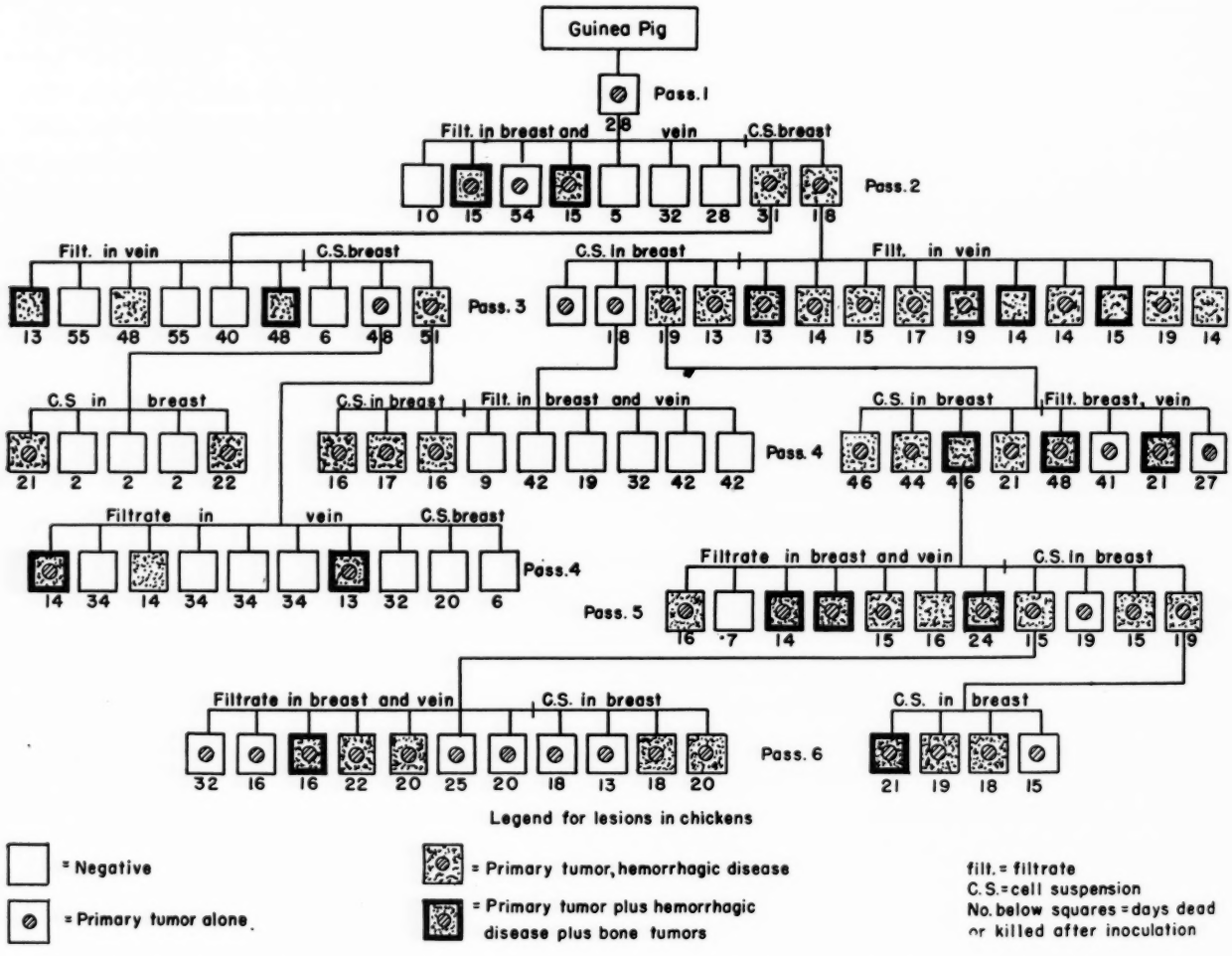


CHART I.—Distribution of various lesions in chickens in a portion of the birds inoculated with the Rous ACT II strain.

TABLE II: INCIDENCE OF BONE LESIONS IN CHICKS INOCULATED WITH ROUS ACT II FILTRATE

	Number of passages					
	1	2	3	4	5	6
Number of chicks	0	23	16	18	7	8
Number with bone lesions	0	3	6	5	3	1

TABLE III: INCIDENCE OF ROUS ACT III TUMORS IN DUCKS IN VARIOUS SERIAL PASSAGES

Passage	Total no. injected	Total no. with tumors
1	3	3
2	5	2
3	10	6
4	3	3
5	4	4

However, the data in Table III suggest that it might have been possible to maintain ACT III in ducks for some time, if not indefinitely.

Stock Rous controls.—The transfer of the Rous sarcoma into guinea pigs was controlled by coincident passage directly into chicks of portions of the tumor used in the pig inoculation. The resulting avian growths were passed in chicks by cell suspensions and Berkefeld "N" filtrates in a manner similar to the inoculated intravenously with the stock Rous showed typical periosteal tumors, identical in type with those found in the ACT strains. These birds were among those in passages 1, 2, and 5 respectively. In no instance were bone lesions found in chicks injected intramuscularly with cell suspensions of the stock Rous sarcoma. This is the first time that periosteal growths

have been observed in the stock Rous tumor in this laboratory. Finally, the incidence of failure to infect animals between the ages of 24 hours and 2 weeks with filtrate of the stock Rous controls was 20 per cent, a value falling above that for ACT I and ACT III but not equalling the 44 per cent found with the ACT II strain.

DISCUSSION

It is believed that the Rous sarcoma has actually been grown in the anterior chamber of the guinea pig eye, and that it has not merely survived in this environment over a period of 10 to 12 days. This belief is based upon the facts that there was an abundance of mitotic figures present on histological examination of the transplant from the guinea pig; that the transplants actually increased in size; and, finally, that when the anterior chamber was irrigated with a dilute cell suspension of the tumor, growth resulted from the widely dispersed sarcoma cells. On the other hand, the behavior of the Rous sarcoma in the guinea pig was different from that of mammalian tumors. Whether this difference is due to inherent peculiarities of the tumor, or to the distant relationship of the hosts involved is not known, and only further work can answer this question.

Two points relative to the passage of the Rous sarcoma from the guinea pig back into the chicken require further emphasis. First, it is clear from the occurrence of acute hemorrhagic disease, a nonneoplastic manifestation of the free virus (1), that the virus as well as the tumor cells survived guinea pig passage. Secondly, it is evident that the guinea pig passage modified the Rous virus in such a manner as to alter its tissue affinities, of which the most outstanding was the development of periosteal tumors. These lesions occurred with all the ACT tumor strains and this property of the virus was maintained through 6, 7, and 8 transfers in 3 separate experiments. It has been shown (2, 3) that by growing the Rous sarcoma in different avian species it is possible to modify the virus as is indicated by altered tissue and species specificities upon reinoculation into chickens. Growth of the duck-adapted Rous sarcoma virus in chickens was difficult and even impossible in older birds (2). New tissue specificities were evidenced by the presence of periosteal and endosteal tumors in the chickens injected with this modified virus. While the Rous tumor adapted to turkeys and guinea fowls showed alteration of the virus when reinoculated into chickens, the modifications were of a minor degree as compared with those with the duck-adapted strain (3). This more mildly modified virus showed no change in host specificity over the original Rous sarcoma virus. However, new tissue affinities were evidenced by the formation of bone tumors in chickens.

The frequency of hemorrhagic blebs in the lungs of birds in the present experiment was considered as additional evidence that variation had taken place in the ACT I tumor, since lesions of this nature have rarely been seen in birds inoculated with stock Rous and were not seen at all in the 146 control birds studied in the present work. With the Rous ACT II tumor strain, while the presence of hemorrhagic lesions of the lungs was not frequent, it is important to point out that the infectivity rate for chicks was low when Berkefeld "N" filtrates were used. This fact might suggest some loss of species affinity on the part of the virus for the chicken. The only evidence that variation existed with the ACT III tumor strain was the presence of periosteal tumors. With this strain hemorrhagic disease of the lungs was infrequent, and the infectivity rate for chickens was higher than for the controls.

It would seem, therefore, that the Rous sarcoma virus was altered as a result of growth in the anterior chamber of the guinea pig eye. This variation was not of the same order in the 3 strains of tumor studied. If the strains were arranged in the order of degree of variation, ACT II would probably come first, with its possible alteration in species affinity; next ACT I, with its hemorrhagic lesions of the lung; and finally ACT III, which differed from the controls only in the presence of periosteal tumors.

When these results are interpreted in the light of those previously reported (2, 3), it would seem that the variation obtained in the Rous virus following guinea pig passage might fall between the changes observed when the virus was passed through turkeys and guinea fowls, on the one hand, and ducks on the other. That is, the variation in all 3 ACT strains was more extreme than the turkey-guinea fowl variants, and less extreme than the duck variants. Whether the virus can adapt itself to the guinea pig as it did to turkeys, guinea fowls, and ducks is a subject of present investigation.

The presence of bone tumors in 4 of the control birds demands an explanation. Such lesions were not found in chicks inoculated with the unaltered Rous virus previous to this experiment, despite the fact that many hundreds of birds have been studied over a period of 6 years. There are two possible explanations. First of all, Oberling and Guérin (7) have shown that storage of the virus of fowl leukosis in glycerol at a low temperature results in an alteration of the tissue affinities and pathological manifestations of the infectious agent. It is conceivable that our method of storage by desiccation in the cold has likewise resulted in a change in the properties of the Rous sarcoma virus. If such were the case, the modification was not as extensive as that following guinea pig

passage, since only 4 periosteal lesions developed in the stock animals, and these were a consequence of intravenous inoculation only.

Secondly, the possibility of contamination of the stock Rous tumor with the ACT virus should not be overlooked. Passages of the ACT tumors were made with the same precautions as those employed with the transfer of 6 other infectious tumors in this laboratory, and there has been no evidence of contamination in the past by the use of this technic. It will be recalled that the animals inoculated with ACT and stock Rous were kept together in the same cage. However, birds with different virus tumors have often been kept in the same pens for long periods of time and have never shown cross infection. Finally, if the presence of the bone tumors in the control stock was the result of contamination one would expect the same incidence of bone lesions, together with other manifestations of the altered virus in this stock Rous strain. This was not the case. On this basis, therefore, it seems most probable that the first explanation for the presence of the bone tumors in the stock Rous is the correct one.[1]

SUMMARY AND CONCLUSIONS

The Rous sarcoma has been grown in the anterior chamber of the guinea pig eye. Its behavior differs from that of mammalian tumors in a similar environment in that vascularization occurs in from 48 to 72 hours after inoculation, and the transplant increases in size by 2 or 3 diameters in 2 weeks, after which it will remain quiescent for as long as 6 months. Evidence that the virus as well as the tumor survives guinea pig transfer is given by the fact that chickens inoculated with transplants from guinea pigs developed hemorrhagic disease as well as tumors.

Growth of the Rous sarcoma in the guinea pig resulted in an alteration in the properties of the virus, for when it was inoculated into chickens periosteal tumors developed. Further, hemorrhagic disease occurred in tissues not frequently affected by the stock Rous virus, and finally, there was a suggestion of an alteration of species affinity for the chicken by one variant Rous strain. Since the 3 strains of the modified Rous virus studied showed different properties, it was evident that although the Rous sarcoma was grown in relatively the same environment in the guinea pig the virus varied in different ways. It is considered that these present changes in the virus represent modifications that fall between those occurring after turkey-guinea fowl passages and the duck passages, as previously described (2, 3).

The fact that an avian tumor virus, supposedly strictly species specific, will thrive in a mammal is of importance not only to the cancer problem but also to the general subject of virus infections. The acquisition by this virus of new tissue affinities falls in line with what we know about viruses varying when transplanted into the "unnatural" environment of a new animal or plant species.

[1] Recently acquired evidence also strongly favors the point of view that the Rous virus was altered during the process of storage. Through the kindness of Doctor Albert Claude, of the Rockefeller Institute, a strain of Rous sarcoma was obtained in desiccated form and maintained in chicks under strict isolation here at New Haven. Of the 29 chicks inoculated intravenously with Berkefeld "N" filtrates, 4 have shown typical periosteal bone tumors.

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Metabolic Studies on Leukemic Mice with the Aid of Radioactive Phosphorus*

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Previous studies in the mouse have shown that the uptake and retention of labelled phosphorus (P^{32}) by tumor transplants, including a lymphoma, is greater than that of the other soft tissues (6, 7, 9). These findings, together with the known high uptake of phosphorus by bone and bone marrow, have led to the use of radioactive phosphorus in the treatment of leukemia (1, 2, 8, 10). Since radioactive phosphorus emits only beta rays, the energy of the radiation is expended close to the site of P^{32} retention.

This present communication is an extension of previous work on the metabolism of phosphorus in normal and lymphomatous mice (7, 9).

EXPERIMENTAL

Twenty male Strong A mice, 6 to 20 weeks old, were used. Ten were inoculated with a suspension of lymphoma cells subcutaneously [1] in the region of the axilla on one side only; the other 10 were used as controls. After inoculation, individual mice were placed in separate wire-bottomed cages. All were fed the same amount of food (Purina dog chow) daily. They were weighed at intervals during the experiment and on the basis of weight were divided into 4 representative groups of 5 animals each.

The radiophosphorus was in the form of an isotonic solution of sodium phosphate.

At the time of phosphorus administration, the dose was equal to 13 μc. Five of the tumor mice and 5 controls were injected with 0.5 cc. of this radiophosphorus solution intraperitoneally 2 days after lymphoma inoculation, and 19 days prior to sacrificing them for analysis of the radioactive phosphorus content. The remaining mice were injected 13 days after tumor inoculation, and 8 days prior to the day on which the animals were killed. Both groups were inoculated with lymphoma cells at the same time and sacrificed at the same time, 8 and 19 days after P^{32} administration respectively.

At necropsy the lymph nodes (on the side opposite the site of lymphoma inoculation) muscle, liver, tumor, and bone were removed for analysis of their activity. Each tissue was weighed and assayed separately, with the exception of the lymph nodes, which were pooled for each group of mice and then analyzed. The data are summarized in Table I.

TABLE I: AVERAGE VALUES OBTAINED UPON TUMOR AND CONTROL ANIMALS AT 8 AND 19 DAYS AFTER ADMINISTRATION OF RADIOPHOSPHORUS IN PER CENT RETENTION OF DOSE PER GM. OF TISSUE

	8 days after P^{32} administration		19 days after P^{32} administration	
Tissue	Tumor animals	Control animals	Tumor animals	Control animals
Lymph nodes	.69	.85	.37	.39
Muscle	.79	.83	.41	.51
Liver	1.00	1.23	.56	.54
Tumor	1.22		.47	
Bone	4.9	4.6	5.4	5.5
Balance	.60	.62	.41	.40
Whole animal	.63	.68	.46	.42

RESULTS

At 8 and 19 days after the administration of radioactive phosphorus, the retention per gram of wet weight of tumor was higher than its analogue, lymph node. These greater retention values for tumor tissue agree with those reported for periods from 1 to 5 days after the administration of a tagged sample of radioactive phosphorus (6, 7, 9). The retention values obtained from lymph node, muscle, liver, and bone are not significantly different when the groups of tumor animals at 8 and 19 days after P^{32} administration are compared with the control animals. The fact that the lymph nodes of the lymphomatous animals did not exceed the controls is suggestive that there was little or no infiltration of lymphoma cells into these nodes.

With respect to the whole animals, the retention of P^{32} is the same in both lymphomatous and control animals at 8 and 19 days after P^{32} administration. These results are similar to those observed 1 to 5 days after

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[1] This lymphoma metastasizes extensively and many of its cells are present in the blood, so that the general condition of the mice resembles leukemia. It was inoculated as a suspension in Ringer's solution, a measured volume (0.03 cc.) of which was injected where lymphomas were desired (dose, 24,300,000 cells).

P^{32} administration to similar groups of mice (9). The phosphorus balance of tumor and normal animals with respect to phosphorus distribution in either the whole body or specific tissues appears to be the same.

DISCUSSION

With the data presented in this and other papers (7, 9) the retention values of tumor, lymph node, liver, muscle, bone, and whole animal are known for a period of 1 to 19 days after P^{32} administration. In interpreting values as indicators of the phosphorus metabolism of each particular tissue, it is desirable to consider what part of the phosphorus retention of each tissue is the result of the characteristics of the tissue itself, and what part is regulated through the activity of other organ systems in the body; namely, those that control the absorption and excretion of phosphorus.

The rate of excretion of P^{32} for the whole animal after the initial uptake necessarily controls the amount of P^{32} that any tissue may contain. After maximum uptake of P^{32}, a tissue actually loses more of the tracer dose than it gains. During and after maximum uptake of P^{32}, the tissues of the body are continually gaining and losing phosphorus through their metabolic activity. However, the chances of gaining a tagged atom for one that is lost become increasingly less because of the continuous excretion of the labelled dose after administration. Thus the retention curves of any tissue must be influenced by: (a) the net tissue exchange of phosphorus and (b) the availability of tagged phosphorus to the tissue, which is of course modulated at any time by the relative quantity that has been excreted. If tissue retention values are corrected for body excretion, they may be more indicative of the intrinsic phosphorus metabolism of various tissues.

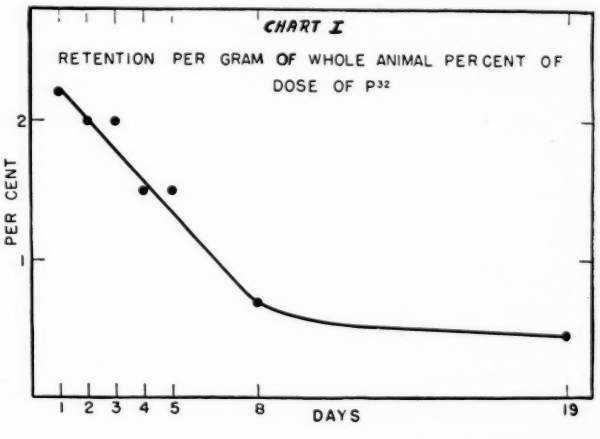
The whole body excretion of P^{32} of these mice and the mice in the previous experiments (7, 9) is summarized as a composite retention curve on 140 mice for a period of 19 days (see Chart I). Excretion data include both normal and tumor mice. The amount of any tagged sample excreted by the whole body was computed from the percentage of the total administered activity remaining in the animals at various time periods. Animals were assayed for activity 1, 2, 3, 4, 5, 8, and 19 days after P^{32} administration. The experimental factors necessary to correct the observed tissue values for body excretion were calculated as follows:

$$\frac{\text{Retention in per cent of dose per gm. of whole body at 1 day}}{\text{Retention in per cent of dose per gm. of whole body at } x \text{ days}} = \text{factor}$$

Since the total retention of P^{32} by normal or lymphomatous mice appears to fall within the same limits, one set of factors is used for both groups of mice. Hence the factor for 1 day is 1.0, 2 days is 1.1, 5 days is 1.7, 8 days is 3.4, and 19 days is 5.0.

The relative retention values are presented in Table II after the values obtained at various time periods on 140 mice were corrected for excretion. These values more accurately represent the exchange of phosphorus between the various tissues of the body when corrected for excretion by the method described above.

When these corrections are made it can be seen that muscle reaches an equilibrium with the tagged sample of phosphorus soon after P^{32} administration (Table II). The phosphorus exchange and equilibrium of muscle in normal and lymphomatous mice is about the same. This appears to be true of liver also in tumor and control animals.



During the time of the experiment bone apparently has not reached equilibrium but continues to gain relatively more of the tagged sample than it loses for the first 19 days after phosphorus administration. This can possibly be explained by the fact that all the phosphorus in bone is not readily available for admixture with other body phosphorus in a short period of time.

The maximum relative uptake of P^{32} by lymph node is observed 5 days after administration. This value gradually decreases with time (Table II). The data obtained for tumor cells and infiltrated lymph nodes are the same as those for lymph node. Both these tissues lose relatively more tagged phosphorus than they gain as time elapses. The relative phosphorus content of lymphoma would be expected to decrease eventually because of the continuous cell division, and growth of this tissue would dilute existing P^{32}.

The intensity of the total phosphorus exchange of these tissues can be demonstrated by comparing the phosphorus content to the maximal relative uptake of tagged phosphorus atoms per unit of tissue phosphorus

(4, 5). The specific activity of tissues at their maximum relative P^{32} retention is as follows:

$$\frac{\text{The retention of dose in per cent per gm. of tissue at maximum value}}{\text{Mgm. of phosphorus per gm. of tissue}}$$

The value obtained is the retention of dose per mgm. of phosphorus in each tissue. These are: muscle, 1 per cent of the dose per mgm. of phosphorus; liver, 0.787 per cent, and lymph node, 0.705 per cent. The

TABLE II: PER CENT RETENTION OF RADIOPHOSPHORUS BY TISSUES AFTER CORRECTION FOR EXCRETION

Tissue		Days after radiophosphorus administration						
		1	2	3	4	5	8	19
Muscle	Control mice	2.1	1.9	2.4	2.0	2.3	2.4	2.2
	Lymphomatous mice	1.8	2.0	2.2	2.0	2.4	2.3	2.1
Liver	Control mice	4.0	3.1	3.2	2.7	3.4	4.2	2.6
	Lymphomatous mice	3.3	3.1	3.2	2.9	4.0	3.4	2.7
Bone	Control mice	5.5	9.8	15.2	9.0	15.6	15.6	27.2
	Lymphomatous mice	4.2	7.6	9.6	12.2	12.4	16.8	26.8
Lymphatic tissue	Control lymph nodes	2.1	2.2		3.5	3.6	3.0	2.0
	Infiltrated lymph node	4.5	5.3	6.1				
	Lymphoma	3.1	3.8	4.4		5.1	4.1	2.6

figures are averages of both normal and lymphomatous mice. With respect to lymphoma, the specific activity is 1.39 per cent of the dose per mgm. of phosphorus. The relative exchange of phosphorus in tumor tissue is about twice that of the normal lymphatic tissue.

CONCLUSIONS

1. Lymphomatous tissue retains more radioactive phosphorus than normal lymphatic tissue from 1 to 19 days after administration of tagged phosphorus.

2. The retention of P^{32} in muscle, liver, bone, and lymph node of lymphomatous mice compares with that of similar tissues in normal mice.

3. The retention time of phosphorus atoms in lymphomatous and normal mice is comparable.

4. When the retention values of muscle and liver are corrected for the excretion of phosphorus by the body, they suggest a quasi-equilibrium with body P^{32} content.

5. Bone continues to gain relatively more tagged dose of phosphorus with the passage of time.

6. The exchange (specific activity) of phosphorus in the lymphoma used here is relatively twice as great as in normal lymphatic tissue.

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The Incidence of Malignant Tumors in British West Indian and Panamanian Negro Autopsy Populations

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Pearl and Bacon (3), Harding and Hankins (1), and other investigators summarized by Lewis (2) feel from their studies that malignant tumors in general affect Negroes much less than the white races. No reports on malignant tumor incidence from this area are available, and it is desired to present the results of such a study upon a reliable cross section of a British West Indian-Panamanian autopsy population.

MATERIAL

The material for this study was obtained from the autopsy protocols of this institution covering the period from January 1, 1929 to June 1, 1944. These autopsy protocols are not from the hospital population alone, but represent a reliable cross section of all people dying in the Canal Zone and many of those dying in the Republic of Panama. The Panama Canal, being a government controlled organization, has one mortuary establishment on each side of the Isthmus, and all persons dying within the limits of the Canal Zone are handled through the respective mortuaries. All dying through traumatic agencies and those dying at home unattended by a physician are routinely autopsied. In all other instances strong representation is made to obtain consent for autopsy examination. During the period studied 62.36 per cent of all white and Negro bodies received in the morgue were autopsied. The percentage of autopsies on British West Indian and Panamanian bodies received is approximately 75 and, from this group, all cases 10 or more years of age form the respective autopsy populations.

Autopsy populations so derived are unique, and represent a reasonably accurate section of the general population without being heavily influenced by hospital cases. The studies presented here are probably more trustworthy, therefore, than other similar hospital series, and much more so than general death registration studies owing to the reliability of the diagnoses. It must be borne in mind that the tumors studied were occasionally incidental findings accompanying other fatal conditions; in each case, however, complete autopsies were performed by competent pathologists and all tumor diagnoses were verified by histologic examination.

Racial determination was made arbitrarily in the following manner, inasmuch as no reliable criteria according to name, color, or appearance are available; persons born of parents born in the British West Indies are classified as British West Indians, whether they were born in Panama or the British West Indies, and the same criteria were applied to the Panamanian group. All members of both autopsy groups were described in the protocols as being either black, brown, or mestizo.

RESULTS

In Table I the race, sex, and age group distributions of the 2,553 cases comprising the autopsy populations are given.

The relative frequency of occurrence of malignant tumors in the autopsy populations according to race, sex, and broad tumor groups are given in Table II. Table III gives the actual number of malignant tumors, arranged according to race, sex, and age groups.

In Table IV the malignant tumors in males are shown arranged according to type of tumor, location, race, and age groups. The same information for females is presented in Table V.

DISCUSSION

The Panamanian autopsy population is too small for reliable consideration alone. The figures obtained show a general malignant tumor incidence of approximately one-half that of the British West Indian autopsy population. This results, in part, from the majority of this group (Table I) falling in the age groups before 41 to 50 years. This group is composed largely of laborers in the Canal Zone and it is impossible to determine degrees of racial mixtures by name, color, or appearance. They live and work under the same conditions as the British West Indians, they have intermarried, and they show the presence of sicklemia in 11.2 per cent of their autopsy population (4). It is proper, therefore, to combine these two autopsy popu-

TABLE I: RACE, SEX, AND AGE DISTRIBUTION OF AUTOPSY POPULATION

Age groups (years)	British West Indians			Panamanians			British West Indian and Panamanian		
	I (male)	II (female)	I and II	I (male)	II (female)	I and II	I (male)	II (female)	I and II
10-15	12	10	22	7	8	15	19	18	37
16-20	38	35	73	28	12	40	66	47	113
21-30	91	65	156	69	30	99	160	95	255
31-40	140	68	208	65	26	91	205	94	299
41-50	423	142	565	57	23	80	480	165	645
51-60	507	136	643	25	11	36	532	147	679
61-70	235	82	317	14	4	18	249	86	335
70 +	118	53	171	10	9	19	128	62	190
Totals	1,564	591	2,155	275	123	398	1,839	714	2,553

TABLE II: THE RELATIVE FREQUENCY OF OCCURRENCE OF MALIGNANT TUMORS IN AUTOPSIED POPULATION

Race	Sex	Total number in autopsied population	Percentage of autopsied population showing: I Carcinomas	II Sarcomas and others	I and II
British West Indians	Male	1,564	12.6	1.6	14.2
	Female	591	13.5	1.7	15.0
	Male and female	2,155	12.8	1.6	14.4
Panamanians	Male	275	1.8	4.0	5.8
	Female	123	6.3	2.4	8.7
	Male and female	398	3.26	3.5	6.78
British West Indians and Panamanians	Male	1,839	11.0	2.0	12.9
	Female	714	12.3	1.8	14.1
	Male and female	2,553	11.3	1.9	13.29

lations in order to obtain the percentage incidence of malignant tumors in this class of person in this area.

Pearl and Bacon (3) analyzed the autopsy populations at Johns Hopkins Hospital and found that malignant tumors, as a group, were much less frequent in Negroes than in the whites. Their figures (Negro and white) included all ages. In order to make comparisons between their report and ours it is necessary to compare separately the figures for the individual age groups. Table VI shows the percentage of malignant tumor incidence in white and Negro autopsies, from their report, compared with the British West Indian-Panamanian Negro group, for specified age groups.

From this table we are in agreement with Pearl and Bacon's conclusions that the incidence of malignant tumors in autopsied Negroes is substantially less than that in autopsied whites, even when a broad general autopsy population is studied so as to lessen the effect of hospital cases. It is necessary to use their figures for the incidence of malignant tumors in autopsied white persons, since the retirement of sick and aged white people by the Panama Canal, with their subsequent return to the United States, makes our autopsy figures on this subject unreliable.

SUMMARY

1. As a result of unique local circumstances in the Canal Zone, the British West Indian-Panamanian Negro autopsy population studied represents a reliable cross section of pathologic findings in these people dying at all ages in this area.

TABLE III: MALIGNANT TUMORS CLASSIFIED BY RACE, SEX, AGE, AND TYPE OF TUMOR

Race	Sex	Type of tumor	10 15	16 20	21 30	31 40	41 50	51 60	61 70	70 +	Totals Male	Totals Female	Totals Male and female
British West Indians	Male	I Carcinoma	1	0	0	11	42	71	53	19	197		277
		II Sarcoma and others	2	1	1	1	7	11	2	0	25		35
		I and II	3	1	1	12	49	82	55	19	222		312
	Female	I Carcinoma	1	0	2	10	24	22	15	6		80	
		II Sarcoma and others	1	0	0	2	2	5	0	0		10	
		I and II	2	0	2	12	26	27	15	6		90	
Panamanians	Male	I Carcinoma	0	0	1	1	2	1	0	0	5		13
		II Sarcoma and others	2	1	4	2	1	0	0	1	11		14
		I and II	2	1	5	3	3	1	0	1	16		27
	Female	I Carcinoma	0	0	0	4	2	1	0	1		8	
		II Sarcoma and others	0	0	1	1	0	0	1	0		3	
		I and II	0	0	1	5	2	1	1	1		11	

TABLE IV: MALIGNANT TUMORS ACCORDING TO RACE AND AGE IN MALE AUTOPSY POPULATION

Type of malignant tumor	British West Indian									Panamanian								
	10–15	16–20	21–30	31–40	41–50	51–60	61–70	70+	Tumor totals	10–15	16–20	21–30	31–40	41–50	51–60	61–70	70+	Tumor totals
Carcinoma of																		
Skin				1	2	1			4									
Breast							1		1									
Mouth					2	2	1	1	6									
Jaw (adamantinoma)					1	2			3									
Tongue					1	3	1	1	6					1				1
Frontal sinus						1			1									
Larynx					1	1	1		3						1			1
Esophagus				4	11	8	9	3	35									
Stomach				1	8	15	15	1	40				1	1				2
Colon and rectum	1				2	3	3	1	10									
Pancreas				2	2	5	1		10									
Liver					5	4	3		12			1						1
Gall bladder							1		1									
Ampulla of Vater						2			2									
Kidney							1		1									
Urinary bladder					3	8	2	1	14									
Prostate				1	4	10	10	11	36									
Testis						1	1		2									
Adrenal						2			2									
Lung				2		2	3		7									
Pituitary						1			1									
Sarcoma and others																		
Lymphosarcoma					1	3			4	1	1	2						4
Liposarcoma														1				1
Chondrosarcoma	1								1									
Rhabdomyosarcoma	1								1									
Kaposi sarcoma					1		1		2				1					1
Endothelioma						1			1									
Hodgkin's sarcoma					1		1		2	1								1
Leukemia, myeloid			1						1			2	1					3
Multiple myeloma						4			4									
Ewing tumor		1							1									
Brain tumor				1	4	1			6								1	1
Malignant melanoma						2			2									
									222									16

TABLE V: MALIGNANT TUMORS ACCORDING TO RACE AND AGE IN FEMALE AUTOPSY POPULATION

Type of malignant tumor	British West Indian									Panamanian								
	10 15	16 20	21 30	31 40	41 50	51 60	61 70	70 +	Tumor totals	10 15	16 20	21 30	31 40	41 50	51 60	61 70	70 +	Tumor totals
Carcinoma of																		
Breast				2	9	6			17									
Larynx					1				1									
Esophagus				1		2	2		5									
Stomach				1	1	1	1	1	5					1				1
Jejunum							1		1									
Colon and rectum				1	1	1	1		4				1					1
Urinary bladder							2		2									
Ovary													1					1
Endometrium					1	2	1	1	5									
Cervix			2	5	5	7	5	1	25				1					1
Vulva and clitoris					3				3									
Pancreas					1		1		2					1	1			2
Liver	1					1			2									
Gall bladder					1	1			2								1	1
Lung					1	1	1	2	5									
Pituitary								1	1									
Chorion (choriocarcinoma)													1					1
Sarcoma and others																		
Osteosarcoma	1								1									
Neurosarcoma					1				1									
Lymphosarcoma				1					1									
Myosarcoma (uterus)						1			1									
Leukemia, myeloid													1					1
Multiple myeloma					1				1									
Brain				1		3			4			1				1		2
Endothelioma						1			1									
									90									11

TABLE VI: PERCENTAGE OF AUTOPSIED CASES IN BALTIMORE * AND IN THE CANAL ZONE SHOWING MALIGNANT TUMORS

Age groups	Male			Females		
	Baltimore		Canal Zone, British W. I., & Pan.	Baltimore		Canal Zone, British W. I., & Pan.
	Whites	Negroes	Negroes	Whites	Negroes	Negroes
10-40	11.9	3.3	6.2	13.0	3.8	8.7
41-50	19.9	9.2	10.8	29.3	19.6	17.0
51-60	24.0	13.1	15.6	38.8	24.0	19.0
61-70	29.2	13.7	22.1	44.6	†	18.6
70 +	18.7	†	15.6	†	†	†
All ages	19.3	7.4	12.9	24.0	14.1	14.1

* From Pearl and Bacon, cited in text.
†Fewer than 10 deaths.

2. Malignant tumors occurred in 339 instances in 2,553 cases 10 or more years of age, or 13.29 per cent.

3. Malignant tumors occurred in 238 instances in 1,839 males, or 12.9 per cent; for females there were 101 tumors in 714 cases, or 14.1 per cent.

4. Carcinoma was 5 times as frequent as sarcoma and other malignant tumor types.

5. Malignant tumors in this Negro autopsy population, when compared according to sex-age groups with other reports, show essential similarity, and additional support is presented for the belief that malignant tumors as a group tend to occur less frequently in Negroes than in the white races, even when living in environments different from the United States.

6. The type of tumor, location, and age incidence are given in tabular form.

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3. PEARL, R., and BACON, AGNES L. Biometrical Studies in Pathology. V. The Racial and Age Incidence of Cancer and of Other Malignant Tumors. Arch. Path., **3**:963-992. 1927.
4. TOMLINSON, W. J. The Incidence of Sicklemia and Sickle Cell Anemia in 3000 Canal Zone Examinations upon Natives of Central America. Am. J. M. Sc., **209**:181-186. 1945.

Abstracts

Reports of Research

Preparation of Stable Colloidal Solutions of Carcinogenic and other Water-Insoluble Compounds. FEIGENBAUM, J. [Hebrew Univ., Jerusalem, Palestine] *Nature, London,* **155**:207. 1945.

A solution of carcinogen (*e.g.* methylcholanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene) in acetone is added drop by drop to distilled water, and the mixture is then dialyzed in a cellophane bag against distilled water for 2 or 3 hours to free it from acetone. Stable colloidal solutions of "more than 1% concentration" can be prepared.—E. L. K.

9,10-Dimethyl-1,2-Benzanthracene as a Highly Potent Carcinogen for the Rabbit's Skin. BERENBLUM, I. [Oxford Univ. Research Centre of Brit. Emp. Cancer Campaign, Oxford, England] *Cancer Research,* **5**:265-268. 1945.

9,10-Dimethyl-1,2-benzanthracene is highly carcinogenic for the rabbit's skin, producing multiple, progressively growing warts after 5 weeks' application, and malignant tumors after about 16 weeks' application. By comparison 3,4-benzpyrene is a very weak carcinogen for the rabbit's skin, while tar, though fairly potent in the sense of inducing early warts, is relatively weak when judged on the basis of continued growth and development of malignancy.—Author's summary.

Epithelial Tumours of the Urinary Bladder in Mice Induced by 2-Acetyl-amino-fluorene. ARMSTRONG, E. C., and BONSER, G. M. [Univ. of Leeds, Leeds, England] *J. Path. & Bact.,* **56**:507-512. 1944.

A suspension in olive oil of 2-acetylaminofluorene was given by stomach tube thrice weekly to 17 CBA mice, of which 12 lived for more than 1 month. The highest total dosage was 1 gm. in 65 weeks. Of 6 mice (1 male, 5 females) treated for more than 1 year, 4 females and the male developed primary neoplasms of the bladder. Other tumors were a fibromyoma, and a sarcoma, of the uterus; and hepatomas (in 3 males and 2 females) coexistent in 3 cases with vesical neoplasms. There were no mammary tumors. Spontaneous tumors of the liver and the uterus, but not of the bladder, may occur in this strain. The bladder tumors were (1) in the male, generalized benign transitional cell papillomatosis practically filling the vesical cavity; (2) similar to (1), with apparent downgrowth; (3) papilloma with malignant subepithelial downgrowth; (4) transitional cell carcinoma penetrating through muscularis to serosa; (5) "massive infiltrating carcinoma completely breaching the muscular wall." Thus only (1) was considered to show no malignant portions. No metastases were found. Ten photomicrographs are included.—E. L. K.

Der Metallkrebs. Ein neues Prinzip der Krebserzeugung. [Metal Cancer: A New Principle in Carcinogenesis.] SCHINZ, H. R., and UEHLINGER, E. [St. Gallen Canton Hosp., and Zurich Univ., Zurich, Switzerland] *Ztschr. f. Krebsforsch.,* **52**:425-437. 1942. From abstr. in *Chem. Zentralbl.,* **I**:401. 1943.

A fuller report than appeared in *Schweiz. med. Wchnschr.,* **72**:1070. 1942; abstr. in *Cancer Research,* **4**:654. 1944. Of 12 rabbits living more than 3 years after implantation of chromium, arsenic, or cobalt into the femur, 8 developed cancer at the site of the depot or in the lungs.—M. H. P.

Über die Anreicherung und Spaltung der Abderhaldenschen Abwehrfermente bei Carcinomkranken. [Concentration and Cleavage of Abderhalden's Protective Enzymes in Carcinoma Patients.] HINSBERG, K., and SCHLEINZER, B. [Path. Inst., Berlin Univ., Berlin, Germany] *Ztschr. f. Krebsforsch.,* **53**:35-46. 1942. From abstr. in *Chem. Zentralbl.,* **I**:166. 1943.

Abderhalden's protective enzymes from the urine of patients with carcinoma were separated into a protein and a low molecular weight fraction by treatment in a circulating dialyzer by Manegold's method (*Kolloid-Ztschr.,* **56**:7. 1931), preferably at pH 5.5 to 6, for 2 to 3 days. After neutralization neither the dialyzate nor the material remaining within the dialyzing apparatus showed enzymic activity, but when the 2 substances were mixed, activity was evident. The inner liquid was thermolabile, the outer, stable to heating at 100° C. for 30 minutes. The authors postulated that the separation was into coenzyme and apoenzyme. Since, with many such pairs of enzymes, the action specificity is determined by the coenzyme, and the substrate specificity, by the apoenzyme, an attempt was made to see whether this was the case with regard to the protective enzymes. Investigations in which coenzyme and apoenzyme were interchanged, however, had only slight success, since the specificity became lost as the protective enzyme solution was concentrated. The concentrated enzyme solution cleaved all carcinoma substrates nonspecifically. Strong cleavage was obtained by addition of 1:20,000 to 1:100,000 trypsin (itself inactive) to apparently inactive defense proteinases.—M. H. P.

Weiterer Beitrag zum Problem des Vorkommens von *d*-Polypeptidase im Blutserum. [Further Contribution to the Problem of the Occurrence of *d*-Polypeptidase in Blood Serum.] ABDERHALDEN, E., and ABDERHALDEN, R. [Physiol. Inst., Martin Luther Univ., Halle, Germany] *Ztschr. f. physiol. Chem.,* **270**:1-8. 1941.

Waldschmidt-Leitz and his associates have proposed the

use of the test for *d*-peptidases as a diagnostic method in cancer. The present authors oppose this. Of 14 sera from patients without carcinoma, 2 attacked *d*-alanylglycylglycine, whereas of 16 sera from patients with carcinoma, 5 hydrolyzed this tripeptide. The peptidase action was measured with the aid of the Willstätter titration. If the colorimetric Zimmermann method, employing *o*-phthaldialdehyde, was used as recommended by Waldschmidt-Leitz, the number of positive reactions increased to 11 in the carcinoma sera group and to 6 in the control group. The Zimmermann reagent is considered unsuitable for quantitative following of the cleavage of polypeptides.—K. G. S.

Über die Spaltung der *d*-Peptide durch Krebssera. [The Cleavage of *d*-Peptides by Cancer Serums.] Boretti, G. [Cancer Inst., Milan, Italy] *Ztschr. f. Krebsforsch.*, **52**:438-442. 1942. From abstr. in *Chem. Zentralbl.*, **I**:401. 1943.

d-Leucylglycine was hydrolyzed 10% by serum from a patient with keloid, not at all by serum from normal or cancerous persons, nor by serum from normal or benzpyrene-treated rats. Ultraviolet and roentgen irradiation gave no consistent results with regard to the appearance of ability to cleave *d*-peptides. The cleavage was studied by the alcohol titration method of Waldschmidt-Leitz (*Angew. Chem.*, **55**:324. 1938).—M. H. P.

Über den Cholesteringehalt des Harns von Geschwulstkranken. [Cholesterol Content of the Urine of Patients with Tumors.] Trappe, W. [Path. Inst., Berlin Univ., Berlin, Germany] *Ztschr. f. Krebsforsch.*, **53**: 47-56. 1942. From abstr. in *Chem. Zentrabl.*, **I**:284. 1943.

No correlation appeared between the presence of a malignant tumor and the cholesterol content of the urine, as determined by two methods. Cholesterol esters were found in the urine only of patients with renal disease.—M. H. P.

A Possible Relationship in Animals between Tumor Susceptibility and Stability of Tissue Proteins. Orekhovich, V. N. *Am. Rev. Soviet Med.*, **1**:517-531. 1944.

Beginning with the 19th or 20th day after implantation of the Jensen sarcoma, when a pause in the rate of tumor growth generally occurred, the muscle protein of the sarcomatous rats was split more readily by cathepsin from the tumors, or by extracts of the livers of sarcomatous rats, than was the muscle protein of healthy controls. The muscle proteins of 9 rats with extraordinary resistance to Jensen sarcoma (each rat having resisted 4 successive transplantations) were hydrolyzed much less readily than those of susceptible animals. On the fourth day after transplantation, the skin proteins of some rats showed greater proteolysis by liver cathepsin than did the skin proteins of healthy animals; subsequently the cleavage of skin proteins of the sarcomatous animals showed an inhibition, rather than an augmentation. A similar inhibition was noted in the skin proteolysis in rats inoculated with Krichevski-Sinelnikov sarcoma. In some cases of rapid growth of Jensen sarcoma, there was a pronounced increase of skin proteolysis on the 14th day; in cases of very slow tumor growth, an increase in protein breakdown did not occur even 3 months after transplantation. The cleavage of proteins of whole blood, serum, and plasma by liver enzymes was much lower in cancer-bearing animals and human beings than in healthy controls. Ultraviolet irradiation increased skin proteolysis in healthy and tumor-bearing rats, and in 3 of 5 tumor-resistant rats. When 40 rats were inoculated with Jensen sarcoma, then freed surgically from the developed tumors, and reinoculated 6 to 8 days later with the same type of neoplasm, 14 rats proved resistant to the second implantation, and 9 of these 14 also resisted effects by ultraviolet irradiation upon skin proteolysis. Subcutaneous injection of 3 mgm. of methylcholanthrene in petrolatum considerably decreased skin proteolysis in 10 of 16 rats; benzpyrene, a less active carcinogen, was also less active in inhibiting skin proteolysis, and dibenzanthracene, weakest carcinogen of the 3, did not affect skin proteolysis in doses of 6 mgm.—M. H. P.

Metabolic Studies in Patients with Cancer of the Gastro-Intestinal Tract. XX. Lipotropic Properties of Protein. Abels, J. C., Ariel, I. M., Pack, G. T., and Rhoads, C. P. [Memorial Hosp., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **56**:62-63. 1944.

Previous studies have shown that patients with gastrointestinal cancer suffer from hepatic dysfunction. This study was undertaken to see if the return toward normal hepatic function induced by the feeding of high protein diets was associated with the restoration of a normal chemical composition of the liver. Seven patients with gastrointestinal cancer were given 2.5 gm. of complete protein per kgm. per day for from 10 to 21 days. At laparotomy, the concentrations of fat in their livers were all within normal limits. Nine patients with gastrointestinal cancer who received 75 gm. of amino acids during the 10 hours before operation were found at laparotomy to have liver fat concentrations as abnormally high as those of fasted patients.—M. B.

Thymonucleic Acid in Tumors. Stowell, R. E. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research*, **5**:283-294. 1945.

The evidence is reviewed that shows that thymonucleic acid plays an important role in normal cells and that some tumors have a disturbed balance of nucleic acids. Thymonucleic acid is combined with protein to form the nucleoprotein of chromatin. It plays an important part in the transmission of hereditary characteristics by the genes, in mitosis, in nucleic acid synthesis and balance, and in the protein synthesis of the cell.

Observations on tumors, by the use of macrochemical methods of analysis of thymonucleic acid or visual inspection of Feulgen stained material, are contradictory and inconclusive. Photometric histochemical observations by means of the Feulgen reaction have shown that some epidermoid carcinomas of mice and human beings, and leukemic blood cells of patients contain increased amounts of thymonucleic acid.

Cytochemical studies indicate that the cytoplasm of malignant cells contains increased amounts of ribonucleic acids and suggest that the heterochromatic region of the chromatin plays a specific role in carcinogenesis. The observations in tumors of stickiness, non-disjunction, displacement, and clumping of chromosomes, of polyploid

cells with increased number and volume of chromosomes, of more frequent mitoses, of enlarged nucleoli, and of multinucleate and giant cells are cytologic evidence of abnormalities of nucleic acids in neoplastic cells.

Extracts of cells containing nucleic acids and their breakdown products have a growth-promoting effect on other cells. That thymonucleic acid may induce a specific, predictable change transmissible to subsequent generations of cells has been shown in work with pneumococci. The Shope papilloma virus and the mammary tumor-inciting milk factor of mice contain nucleic acids.

Thymonucleic acid is located in the regions of the chomosome that are most susceptible to mutation. Similar wave lengths of ultraviolet light produce a breaking down of the polymerized sodium thymonucleate, mutations in chromosomes, and carcinoma of the skin. Somatic mutations leading to neoplasia might be produced by alterations in the complex macromolecule of thymonucleic acid. An initial slight modification in the thymonucleoprotein could, during a variable latent period, lead to a progressive and ultimately irreversible imbalance of nucleic acids. Such a theory of an intracellular cause of neoplasia will be established or disproved by subsequent investigation.—Author's abstract.

The Relative Thymonucleic Acid Content of Human Normal Epidermis, Hyperplastic Epidermis, and Epidermoid Carcinomas. STOWELL, R. E., and COOPER, Z. K. [Washington Univ. Sch. of Med., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research,* **5**:295-301. 1945.

A photometric histochemical technic was employed, in which the amount of thymonucleic acid was determined by the absorption of complementary monochromatic light by sections of tissue stained by the Feulgen reaction. Comparative measurements were made on the adjacent normal epidermis, hyperplastic epidermis, and epidermoid carcinomas of 11 specimens removed from patients.

The mean amounts of thymonucleic acid per unit volume of tissue were larger in carcinomas than in normal epidermis and were least in hyperplastic epidermis in which there is relatively less nuclear material. Compared with normal and hyperplastic epithelium, the nuclei of some carcinomas showed statistically significant increases in amount of thymonucleic acid per cell; in no instance was the amount significantly decreased. Such variations in the mean amount of thymonucleic acid in the cells of epidermoid carcinomas support the hypothesis that in some types of neoplasia there is a disturbance of the nucleic acids—Authors' abstract.

Respiratory Behavior of Bacteria-Free Crown-Gall Tissues. WHITE, P. R. [Rockefeller Inst., Princeton, N. J.] *Cancer Research,* **5**:302-311. 1945.

Respiratory studies of a variety of healthy tissues of *Helianthus annuus,* of bacteria-containing tumor tissues, bacteria-free secondary tumors, graft tumors, and tissue cultures of the same plant, and of genetically tumefacient tissue cultures and graft-induced tumors on *Nicotiana* have led to the conclusion that these various pathological states do not result in any apparent significant *qualitative* change in the respiratory picture, but do result in a considerable lowering of the respiratory level. If this lowering is real and not merely an artefact due to the greater amount of nonliving tissue present in pathological growths, it may be considered similar in kind to long recognized characteristics of animal neoplasia.—Author's abstract.

Einfluss von Hormonpräparaten auf den Brenztraubensäure-Gehalt des Blutes und die Geschwulstentwicklung bei sarkomtragenden Ratten. [Influence of Hormone Preparations on the Pyruvic Acid Level of the Blood and on Tumor Development in Sarcoma-Bearing Rats.] v. EULER, H., SÄBERG, I., and v. EULER, B. [Vitamin Inst., Stockholm Univ., Stockholm, Sweden] *Ztschr. f. physiol. Chem.,* **270**:125-140. 1941.

When rats were inoculated with Jensen sarcoma in the second and third weeks of pregnancy, a strong inhibition of the growth rate of the tumor was observed. On the other hand, mating of sarcoma-bearing rats had no effect on the development of the tumor.

By the injection of 50 to 200 I.U. of Prolan (prepared from pregnancy urine) an inhibition or regression of the tumor was produced in 5 of 9 males and in 7 of 14 females; spontaneous regression occurred in only 17 of 150 rats. Treatment with Preloban from pituitary glands had no comparable effect. The authors suspect that the activity of the Prolan preparations is due to an as yet unidentified component of the pregnancy urine.

The presence of sarcomas of medium size (8 to 15 gm.) led to an average blood cell sedimentation value of approximately 6 mm. in 1 hour; rats bearing large Jensen sarcomas had sedimentation values of about 17 mm. in 1 hour.

The intramuscular injection of lanthanum nitrate or sodium tungstate increased the sedimentation rate but did not cause a diminution in the size of the tumor, although under other experimental conditions a diminution in tumor size has occurred with the former compound.

Effects of the various preparations on the pyruvic acid level of the blood are tabulated.—K. G. S.

Krebs, Geschlechtlichkeit, Stoffwechsel. [Cancer, Sex, Metabolism.] TRUTTWIN, H. [Vienna, Austria] *Deutsche med. Wchnschr.,* **68**:1093-1096. 1942. From abstr. in *Chem. Zentralbl.,* **1**:38. 1943.

A review.—M. H. P.

The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. I. Observations on the Adrenal Cortex. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **5**:193-202. 1945.

Two groups of mice belonging to the ce strain, from 1 to 13 months of age, were autopsied at monthly intervals. The groups consisted of (a) 26 intact virgin females and (b) 34 gonadectomized females. Ovariectomy was performed when the mice were 1 to 3 days of age. Adrenal cortical carcinomas were found only in the ovariectomized mice. These occurred in 100% of the 21 females 6 to 12 months of age inclusive, and in none of the 26 intact mice. Progressive changes in the adrenal cortex preceding the appearance of the carcinomas are described.—Authors' abstract.

The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. II. Observations on the Accessory Sex Organs. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **5**:203-210. 1945.

Two groups of mice belonging to the ce strain, from 1 to 13 months of age, were autopsied at monthly intervals. The two groups consisted of (a) 26 intact virgin females, and (b) 34 gonadectomized females. Ovariectomy was performed when the mice were 1 to 3 days old. Growth and development of accessory sex organs occurred after the appearance of adrenal cortical tumors in the ovariectomized females. Effects attributable to estrogenic and to androgenic hormones were recorded.—Authors' abstract.

The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Male Mice of the Extreme Dilution Strain. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **5**:211-219. 1945.

Observations have been made on 33 intact and 39 castrated strain ce male mice. Data were obtained at monthly intervals on animals ranging in age from 1 to 13 months. Adrenal cortical carcinomas were observed in 15 of 19 castrated mice killed at 7 to 12 months of age inclusive. In 4 cases the tumors were bilateral so that the 15 mice carried 19 tumors. No such carcinomas were found in 15 intact strain ce male mice killed during the same age period. The adrenal tumors were preceded by certain definite changes in the cortex, which involved pronounced focal increase in subcapsular cells and later, localized cell hypertrophy. The condition of the accessory sex organs in the castrated mice indicated that they were being subjected to: (a) an estrogenic type of influence in some individuals, and (b) an androgenic type of influence in other individuals. There was some evidence that the difference between individuals was in some cases due only to a variation in proportion of estrogens and androgens, both possibly being present at the same time.—Authors' abstract.

Histological Study of Adrenal Cortical Tumors in Gonadectomized Mice of the ce Strain. FEKETE, E., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **5**:220-226. 1945.

Gonadectomy was performed at 2 days of age on 70 females and 61 males of the extreme dilution ce strain of mice. Adrenal cortical carcinomas arose in many of them. The total percentage of adrenal tumors was 91.90 in the females and 72.54 in the males. There were 31.03% of unilateral adrenal tumors in the females and 54.05% in the males. Bilateral adrenal tumors occurred in 68.96% of the females, and in 45.94% of the males. Only animals more than 3 months old are included in these figures. A histological study with photomicrographs is presented.—Authors' abstract.

The Tumour Virus Disseminated from Rous No. 1 Tumours. CARR, J. G. [Inst. of Animal Genet., Edinburgh Univ., Edinburgh, Scotland] *Proc. Roy. Soc. Edinburgh,* **B., 62**:51-53. 1944.

Of the virus disseminated into the host's tissues from a developing Rous sarcoma, only about 20 minimal infective doses was found in the richest tissue—the spleen. It is suggested that the whole of this can be referred to the amount contained in the blood and phagocytic cells present in the tissues.—A. H.

Lack of Transmission of Avian Tumour Virus from Carrier Hens to their Offspring via the Egg. CARR, J. G. [Inst. of Animal Genet., Edinburgh Univ., Edinburgh, Scotland] *Proc. Roy. Soc. Edinburgh,* **B., 62**:54-58. 1944.

Although hens that are carriers of the Rous sarcoma virus lay eggs that contain a considerable amount of virus-neutralizing antibody in the yolk, virus could not be detected in the eggs, embryos, or chicks derived from such birds, nor did carriers infect other birds in the same pen. The investigation does not suggest that transmission via the egg is an important cause of the high incidence of neoplasms in poultry.—A. H.

Action of Notatin on the Rous No. 1 Sarcoma Virus. CARR, J. G. [Inst. of Animal Genet., Edinburgh Univ., Edinburgh, Scotland] *Nature, London,* **155**:202. 1945.

Addition of the antibiotic substance notatin, 0.2 mgm. together with 2 mgm. of glucose, to 0.5 ml. of a suspension of the Rous agent, equivalent to 1,000 minimum infective doses, resulted in almost complete loss of viral activity in 1½ hours. Notatin without glucose caused merely a slight reduction in the activity of similar suspensions, and glucose alone was without effect. In relation to the known susceptibility of the Rous virus to oxidizing agents, it is pointed out that notatin exerts its antibiotic activity by means of hydrogen peroxide, produced in the oxidation of glucose to gluconic acid.

In contrast with such activity against the Rous agent *in vitro,* no effects upon the virus were observed *in vivo,* when notatin was administered either to fowls bearing the Rous sarcoma or to normal fowls 1½ hours prior to the inoculation of active filtrate.—A. H.

Retardation of the Growth of Mouse Carcinoma 2146 by Histone and Protamine. STEDMAN, EDGAR, STEDMAN, ELLEN, and PETTIGREW, F. W. [Edinburgh Univ., Edinburgh, Scotland] *Biochem. J.,* **38**:xxxi-xxxii. 1944.

An emulsion of this tumor in saline, when injected into the thigh muscles of a mouse, produces a large tumor in 7 days, but if the emulsion is made in 2% histone (from calf thymus, carcinoma 2146, or mouse liver) or protamine (from herring sperm) in saline, the rate of growth is slowed to one-half or less, but the inhibition is transient. Proteins from human plasma, and egg albumin have no such effect.—E. L. K.

The Effects of Extracts of Adult, Embryo, and Tumor Tissues on the Growth of Yeast. COOK, E. S., and WALSH, SISTER T. M. [Inst. Divi Thomae, Cincinnati, Ohio] *Growth,* **8**:251-258. 1944.

The proliferation of *Saccharomyces cerevisiae* in Williams' medium was promoted by saline extracts of mouse embryo, of adult mouse brain, heart, spleen, liver, kidney, and testis, of mouse adenocarcinoma 15091a and dbrB, and of 7-day chick embryos. On an equal weight basis, brain, liver, and embryo were most potent as sources of proliferation-promoting substances, and chick embryo was more potent than adult or embryo mouse tissues. Ultraviolet absorption spectra of 4 of the extracts are

presented. The nature of the active substances is unknown.—M. H. P.

The Heterologous Transplantation of Mouse and Rat Tumors. Greene, H. S. N., and Murphy, E. D. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **5**:269-282. 1945.

A number of mouse and rat tumors including a bronchogenic carcinoma, sarcoma 180, an ovarian embryoma, an experimentally induced hepatoma, 2 mammary carcinomas, and sarcoma 39 were successfully transplanted to certain sites in alien species. The heterotransplantable tumors, in contrast to a group not transferable in this manner, possessed the ability to invade and metastasize in the parent strain and to survive and grow in unrelated strains. On this basis it was concluded: first, that in mice, as well as in man and in the rabbit, invasion marks the attainment of autonomy; and second, that from the viewpoint of autonomy true homologous transfer and heterologous transfer possess the same significance.—Authors' summary.

Sarcomatous Transformation of the Stroma of Mammary Carcinomas That Stimulated Fibroblastic Growth *in Vitro*. Ludford, R. J., and Barlow, H. [Labs. of Imperial Cancer Research Fund, Mill Hill, London, England] *Cancer Research,* **5**:257-264. 1945.

Sarcomatous transformation of the stroma is a common occurrence during the transplantation of mammary carcinomas of high cancer strain mice. The histological evidence of sarcomatous change was confirmed by study of the growth characteristics of tumors *in vitro* before and after they had undergone transformation. In tissue cultures mammary carcinomas exhibited the typical epithelial growth pattern, with few cells of the monocyte-macrophage type, and stimulated fibroblastic growth. The sarcomatous nature of the transformed tumors was indicated by their growth pattern and general cellular morphology, resembling fibroblasts; by their high content of cells of the monocyte-macrophage type; and by their inhibiting fibroblastic growth. Of the factors responsible for the frequency of sarcomatous change in the high mammary cancer strains, special significance is attributed to: (a) the considerable stimulation of fibroblastic growth by the carcinoma cells; and (b) stromal cells surviving transplantation because the cells of the graft are homozygous with those of the new host.—Authors' summary.

The Barnard Free Skin and Cancer Hospital Research Report for 1943. Cowdry, E. V. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *J. Missouri M. A.,* **41**: 181-183. 1944.

The hospital from the beginning has been a center for research in dermatology, and the present report outlines the various phases of research activity being carried on. Progress is reported in the study of the relation between the cutaneously applied carcinogen and early epidermal reactions. The advance has been made possible by the use of fluorescence microscopy.—M. E. H.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

The Relation Between the Incidence and Incubation Period of Cancer in Man. Kennaway, E. L., and Kennaway, N. M. [Chester Beatty Research Inst., Roy. Cancer Hosp., London, England] *Yale J. Biol. & Med.,* **17**:139-161. 1944.

Some data have been collected upon the relation between (a) the total incidence and (b) the incubation period of cancer in man. A more intense stimulus is required to shorten (b) than is necessary to increase (a), and (b) seems to be especially under the influence of genetic factors. Thus, the mean age at death from cancer of the scrotum is almost the same in chimney-sweeps as it is in the general population, although the liability to this disease among sweeps is enormously greater than it is in most other occupations. Even the extremely high incidence of lung cancer among the workers at Griesheim is not accompanied by any early age at which death from this disease occurs. On the other hand, some forms of cancer in which a familial factor is concerned (cancer of the colon and rectum in families showing polyposis intestini, the cancers of the gastrointestinal tract and endometrium in the family "G" of Warthin, some cases of cancer of the breast, xeroderma pigmentosum) lead to death at an age which is, on the average, much earlier than that seen in subjects of similar forms of cancer in the general population, while in these families cancer of other organs occurs at the usual "cancer age."—Authors' summary. (J. L. M.)

Trauma

Single Trauma as an Inciting Factor in Carcinoma. Leighton, W. E. [Barnard Free Skin and Cancer Hosp., and St. Louis Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:994-1002. 1944.

The author analyzes 13 cases of cancer of the penis, seen at the Barnard Free Skin and Cancer Hospital, in every one of which a history of a single trauma to the penis was present and the growth followed all the postulates laid down by Segond as necessary to establish the causal relationship between trauma and the later development of cancer—J. L. M.

Accidental Trauma and Tumor Metastasis. Toth, B. J. [Olean, N. Y.] *Radiology,* **42**:579-590. 1944.

Two cases of generalized metastasis first brought to attention by trauma were carefully studied. In one case the primary tumor was in the lung, in the second, in the stomach. Metastasis developed at the site of the trauma, and apparently all the requirements for indicating a relationship between trauma and tumor were established. However careful study of other metastatic areas showed the same appearance without injury. Deliberate trauma to other areas failed to produce metastasis. It was concluded that there was probably no scientific proof of a causal relationship and that the unknown laws of metastasis influence the localization of secondary deposits.—R. E. S.

Therapy—General

Neurosurgery and Radiation for the Relief of Pain in Advanced Cancer. Cooper, G., and Archer, V. W. [Univ. of Virginia Hosp., University, Va.] *Radiology,* **43**:142-146. 1944.

Patients with advanced or metastatic cancer should be given the benefit of irradiation or neurosurgery since palliation can frequently be obtained with relief of pain and prolongation of life. Seven illustrative cases are described.—R. E. S.

Acute Surgical Conditions Complicating Malignancy. Rawls, J. L. [Norfolk, Va.] *Virginia M. Monthly,* **71**:232-234. 1944.

Presentation of several cases to combat the idea that a cancer patient is not entitled to treatment for an intercurrent condition requiring surgery.—M. E. H.

Castration in Malignant and Non-Malignant Disease. Orndoff, B. H. [Loyola Univ. Sch. of Med., Chicago, Ill.] *Radiology,* **42**:159-164. 1944.

The beneficial effects of castration of the male in carcinoma of the prostate are discussed together with the changes in androgen output after castration by surgery or irradiation. In the female, castration seems indicated in carcinoma of the breast when there is associated pregnancy. A plan of treatment is offered for such cases, which includes: interruption of pregnancy by x-ray followed if necessary by surgery; in early cases, preoperative radiation to the breast, followed by radical surgery and postoperative irradiation; continuation of pelvic irradiation at intervals of 60 to 90 days until all symptoms of the climacterium have ceased, or for at least 2 years; and administration of androgen, *e.g.,* testosterone, during preoperative irradiation and at intervals postoperatively.—R. E. S.

Skin and Subcutaneous Tissues

Treatment of Vascular Nevi. Anderson, C. R. [Los Angeles, Calif.] *J. Pediat.,* **25**:148-149. 1944.

Vascular nevi of infancy should not be treated before the patient is 6 years of age since spontaneous involution will have occurred in the majority of lesions by that time. X-ray and radium have no place in the therapy of these lesions.—M. E. H.

Epithelioma Adenoides Cysticum (Naevus folliculari s of Brook). Whittle, C. H. *Proc. Roy. Soc. Med.,* **37**: 415-416. 1944.

Report of a case in a woman aged 31. There were multiple lesions confined to the upper lip and forehead. Biopsy tissue showed laminated keratin distending the upper part of a follicle. There was some hyperplasia of basal cells arranged in palisades suggesting an attempt at the formation of hair follicles. The upper lip is severely affected in this condition as distinct from adenoma sebaceum. The Brook's tumor is a frequent precursor of basal celled carcinoma.—L. W. P.

Dermatofibromyxosarcoma. McClean, E. D., and Pugh, P. F. H. [Broadlawns Gen. Hosp., Des Moines, Iowa] *J. Iowa M. Soc.,* **34**:352-354. 1944.

Report of a typical case of a rare tumor. Early surgery is the treatment of choice.—M. E. H.

Glomus Tumors: Diagnosis and Treatment. Love, J. G. [Mayo Clin., Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **19**:113-116. 1944.

This tumor is not yet well known; it is frequently overlooked and the patient considered to be neurotic or a malingerer. The author calls attention to his so-called "pin" test, which is based on the supersensitivity of the tumor to light tactile stimulation. He has found the test to be valuable not only in the diagnosis but in the localization of the tumor. The treatment of this lesion is surgical excision.—J. L. M.

A Mixed Tumor of the Salivary Gland Type on the Left Hand. Highman, B. [Nat. Inst. of Health, Bethesda, Md.] *Arch. Path.,* **37**:387-388. 1944.

Case report, with tabulation of the findings in 10 similar cases collected from the literature. Trauma is suggested as a possible predisposing factor.—J. G. K.

Rodent Ulcer Treated by Application of Sodium Bicarbonate. Cameron, D. [Cumberland Infirmary, Cumberland, England] *Lancet,* **247**:720-722. 1944.

Twenty-eight malignant growths of the face and neck, of which 24 were examined microscopically, occurring in 24 patients, were treated by the application of sodium bicarbonate as a saturated watery solution, or as various mixtures of this solution with glycerin, or as ointments, which were less efficacious (15 to 30% in lanolin, eucerin, or soft paraffin). Successful treatment required from 10 days to 10 months. Eight of 16 uncomplicated rodent ulcers, verified histologically, disappeared; of these, 4 have been healed for more than 5 years, one for more than 3 years, and 3 for about a year. Two of the lesions cured were considered to be, in part, epitheliomas. Full histories of 11 cases, 2 photographs, and 3 photomicrographs are included.—E. L. K.

Eye

The Removal of Malignant Tumours of the Iris. Juler, F. A. *Proc. Roy. Soc. Med.,* **37**:689-692. 1944.

A description of 2 cases with a discussion of the signs of malignancy and of the possible methods of treatment.—E. L. K.

I. Metastatic Carcinoma of the Choroid. II. General Metastasis from a Melanoma of the Abdominal Wall, with Paresis of the External-Rectus Muscle. III. Rubeosis Iridis, with Melanoma of the Choroid and Secondary Glaucoma. Ellett, E. C. [Memphis, Tenn.] *Am. J. Ophth.,* **27**:726-731. 1944.

A brief description with case reports of each condition.—E. C. R.

Retro-Orbital Adrenal Rest Tumor. Hughes, L. W., and Ambrose, A. [Newark, N. J.] *J. A. M. A.,* **126**:231-232. 1944.

A case reported because of its apparent rarity.—M. E. H.

Tumor of the Lacrimal Gland. Flick, J. J. [Indiana Univ. Hosp., Indianapolis, Ind.] *Am. J. Ophth.,* **27**: 362-368. 1944.

These rare tumors are usually of the mixed type and when invasive tend to erode through the roof of the orbit. A case in which operation was performed through the intracranial approach is reported.—E. C. R.

Female Genital Tract

Corpus Luteum-Cysts and/or Pregnancy? Nyst, P. M. E. E. [Gouda, Netherlands] *Nederl. tijdschr. v. geneeesk.*, **87**:590-593. 1943. From abstr. in *Chem. Zentralbl.*, **II**:330. 1943.

Description of a case of cystic degeneration of a corpus luteum of pregnancy.—M. H. P.

Adenocarcinoma with Clear Cells (Hypernephroid) of the Ovary. Saphir, O., and Lackner, J. E. [Michael Reese Hosp., Chicago, Ill.] *Surg., Gynec. & Obst.*, **79**:539-543. 1944.

Report of 2 cases. The yellow, malignant growths did not produce masculinization and were different from the lutein cell tumors; they apparently arose from mesonephric structures within the ovary and seemed to be histologically identical with the hypernephroid carcinomas of the kidney.—J. G. K.

On the Oestrogenic Origin of Uterine Fibromyomas. Shute, E. [London, Canada] *Canad. M. A. J.*, **51**:443-445. 1944.

Estrogenic stimulation induces the formation of uterine fibromyomas in certain species of experimental animals. The histories of 130 consecutive, unselected women having fibroid uterine tumors were analyzed. Of these women 52% gave a history of menorrhagia, probably functional, in the second decade; 63% of them were hypothyroid. Both functional menorrhagia and hypothyroidism are generally associated with high estrogen levels in the body. Human fibromyomas may be of estrogenic origin. If true, this offers a clue to their prophylaxis, which should be one of the first aims of gynecology. The prophylactic measures should probably include the use of such anti-estrogens as thyroid extract or vitamin E.—Author's summary. (M. E. H.)

The Value of Periodic Pelvic Examination in the Control of Cancer of the Uterus. Macfarlane, C., Sturgis, M. C., and Fetterman, F. S. [Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *J. A. M. A.*, **126**:877-880. 1944.

Of 1,319 female volunteers between the ages of 30 and 80 years, 416 reported regularly for examination twice a year for 5 years, and a total of 545 more or less intermittently completed the 5 year period. The pelvic examinations were started in 1938; since January of 1942 the breasts have been examined also. In the first examination of 1,319 volunteers, early cancer of the cervix was discovered 3 times and in each instance in areas of papillary erosion. In the total 9,111 examinations, 4 early cancers of the cervix and 1 early cancer of the uterus were discovered; all were successfully treated. Of 461 inflammatory lesions of the cervix discovered, 200 were treated and eliminated. In the course of the work, 18 cancers of 10 different organs were discovered by, or reported to, the authors. "The death rate from cancer of the uterus could be materially reduced by the semiannual pelvic examination of married women 30 years of age and over."—M. E. H.

Carcinoma of the Endometrium. Waterman, G. W. [Rhode Island Hosp., Providence, R. I.] *Rhode Island M. J.*, **27**:577-579; 581-582; 619. 1944.

One hundred and forty cases of cancer of the endometrium are presented for consideration with respect to marital status of the patient, gravidity, age incidence, symptoms, and associated diseases. The 5 year survival rate for 80 cases treated between 1924 and 1938 was 36%. The results are considered from the standpoint of clinical stage and pathological grade, as well as for type of treatment. The author concludes that cancer of the endometrium is essentially a disease to be treated by surgery, *i.e.* panhysterectomy, but that the prognosis can be greatly improved through the use of radium or x-ray as a preliminary to the surgery.—M. E. H.

Anzeigen und Erfolge der Anwendung männlichen Hormons bei Gynäkologischen Leiden. [Indications and Results of the Use of Male Hormone in Gynecological Disorders.] Winkler, H. [Marburg Univ., Marburg a. d. Lahn, Germany] *Therap. d. Gegenw.*, **84**:220-222. 1943. From abstr. in *Chem. Zentralbl.*, **II**:1886. 1943.

Excessive dosage of male sex hormone should be avoided in gynecological practice because of the danger of injuring the ovaries. The benefits of this hormone in glandular cystic hyperplasia are not lasting, according to the author's experience. Questions are also raised concerning the use of large doses in hemorrhage from myoma, and small doses in climacteric disorders, mastodynia, and chronic cystic mastopathia.—M. H. P.

Melanocarcinoma of the Cervix Uteri or Vaginal Vault. Taylor, C. E., and Tuttle, H. K. [Gorgas Hosp., Ancon, Canal Zone] *Arch. Path.*, **38**:60-61. 1944.

Report of a case in which the patient survived 13 years after operation, with multiple local recurrences, and finally died with generalized metastases. No other reports of melanoma arising primarily in this location were found in a search of the literature.—J. G. K.

Pelvic Myofibromas of Extra-Uterine Origin. Strickland, C. G. [Erie, Pa.] *Pennsylvania M. J.*, **47**:489-490. 1944.

Myofibromas in the pelvis may arise in or from structures other than the uterus. These tumors have the same macroscopic and microscopic appearance as the uterine myofibroma and, after removal, are indistinguishable therefrom. Extrauterine myofibromas may occur in association with those of uterine origin, but may also occur in women whose uterus either has been removed or shows no signs of involvement. Two case reports are presented of myofibroma in the vagina and retroperitoneum respectively.—J. L. M.

Male Genital Tract

Present Concepts on the Treatment of Carcinoma of the Prostate. Moore, R. A. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1198-1202. 1944.

A review with special reference to therapy by castration and estrogens.—J. L. M.

Combined Surgical and Hormonal Treatment for Cancer of the Prostate. Rose, D. K. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1203-1210. 1944.

A review of the pathology, diagnosis, and treatment of this disease.—J. L. M.

The Difference between Prostatic Phosphatase and other Acid Phosphatases in Pathological Human Sera. HERBERT, F. K. *Biochem. J.*, **38**:xxiii-xxiv. 1944.

Increased amounts of acid phosphatase in the serum are almost constantly found in prostatic carcinoma when metastases are present and are found also in many cases without demonstrable metastases. Often the increases are so great as to be clearly diagnostic, but in some cases there are only slight increases such as are found occasionally in other diseases. Phosphatase from prostatic tissue differs from other acid phosphatases in being inactivated by incubation for 1 hour at 37° C. at pH 7.4 or by treatment with ethyl alcohol under specified conditions. —E. L. K.

The Clinical Significance of Serum Acid Phosphatase with Especial Reference to Carcinoma of the Prostate Gland. TRAFTON, H., and PERKIN, H. J. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **4**:59-63. 1944.

The correlation of 925 determinations of serum acid phosphatase with clinical observations indicates that the simplified determination method employed provides sufficiently consistent and specific results to be a valuable, but not conclusive, supplement to other procedures in the diagnosis, treatment, and prognosis of prostatic carcinoma. Acid phosphatase values of 0.8 to 1 unit (method of Bodansky, *J. Biol. Chem.*, **101**:93. 1933) suggest the presence of metastasizing carcinoma of the prostate, especially when the alkaline phosphatase is normal. Acid phosphatase values of 1.2 units or more are pathognomonic of carcinoma with bony metastases, especially if the alkaline phosphatase also is elevated. However, a normal acid phosphatase level does not prove that metastases are absent.—M. H. P.

Deux Cancers Leydigiens de l'Homme. Leur Comparaison avec les Tumeurs Interstitielles Expérimentales de la Souris. [Two Cases of Leydig Cell Carcinoma in Man. Their Comparison with Interstitial Cell Tumors Experimentally Obtained in Mice.] MASSON, P. [Montreal Univ., Montreal, Canada] *Rev. canad. de biol.*, **2**:168-243. 1943.

Two cases of interstitial cell carcinoma of the testis, occurring in men of 62 and 32 years, are reported, with autopsy records. Both tumors had almost entirely an endocrine structure and were composed of cells possessing all the essential characteristics of Leydig cells. Histologically they were similar to other interstitial tumors that were reported in the literature as benign or malignant and that, after removal, did not recur or metastasize. It was possible to ascertain the cancerous nature of the tumors described here, only because the patients were followed for several (4 and 9) years.

The first tumor was uninodular and unicentric and seemed to arise from normal Leydig cells. Its cells showed division by mitosis only and metastasized by the lymphatic channels. The second was multinodular and multicentric and contained figures of mitosis and amitosis. It was located in a testicle in which interstitial cells were few, aplastic, and atrophied. Each autonomous tumor nodule was formed of Leydig cells, locally derived from mesenchyme cells by progressive differentiation. This mode of cytogenesis was also evident in the spermatic cord 9 years after removal of the primary lesion. The funicular nodule formed gave rise to the visceral and bone metastases via the blood vessels.

These two human tumors are analogous to the interstitial tumors experimentally produced in mice by the prolonged administration of estrogens. The first case resembles triphenylethylene-provoked tumors in arising from preformed Leydig cells, and the second resembles stilbestrol-induced tumors, which develop from outgrowths of new interstitial cells, mesenchymal in origin, after the normal Leydig cells have disappeared.

The induction and growth of Leydigian tumors in mice and the formation of metastases requires a genetic and a hormonal (*i.e.* a permanent excess of estrogen) factor. The necessity for the genetic factor in man is implied. In the second case reported, there was an abnormally high excretion of estrogens by the kidney. Experimental tumors induced by stilbestrol secrete androgenic substances. The urine of the second patient contained each day, besides an excessive amount of estrogens, 1 gm. or more of androgens (50 times normal). No hormonal assays were made in the first case.—C. A.

Interstitial-Cell Testicular Tumour. NEEVE, R. H., and MARSH, F. [Anglo-Iranian Oil Co., Ltd., Iran] *J. Path. & Bact.*, **56**:575-576. 1944.

The tumor was removed from a Persian, who was in good health a year later. The tumor cells were all of one type and resembled closely those described by Bonser and Hawksley (*J. Path. & Bact.*, **55**:295. 1943; abstr. in *Cancer Research*, **4**:664. 1944).—E. L. K.

Tumors of Testis Following Mumps Orchitis. Case Report and Review of 24 Cases. GILBERT, J. B. [Schenectady, N. Y.] *J. Urol.*, **51**:296-300. 1944.

After a statistical study of reported and personal cases the author concludes there is no direct relationship between mumps orchitis and the development of testicular neoplasia.—V. F. M.

URINARY SYSTEM—MALE AND FEMALE

Kidney Tumors. HOWES, W. E. [Brooklyn, N. Y.] *Radiology*, **42**:319-328. 1944.

A classification, and symptoms, diagnosis, therapy, and end results in 54 cases of carcinoma of the kidney seen at the Brooklyn Cancer Institute are presented. Tumors are divided into those arising in the kidney cortex, in adrenal rests (hypernephroma), and in the kidney pelvis. Papillary adenocarcinoma is the most frequent. Diagnosis may be delayed until metastasis is produced, since the primary growth is often silent. Bone metastases are usually single with little bone reaction. Lung metastases are usually cottony and suggest hematogenous origin. Fourteen of the 54 patients had presenting symptoms arising from metastases. Only 11 patients are now living, and only 5 of these have survived 3½ to 7 years. Preoperative radiation is theoretically worth while, and it was given in a few instances. The primary tumor may be radiosensitive, but the metastases are almost uniformly resistant.—R. E. S.

Malignant Tumors of the Kidney: Review of 117 Cases. Bixler, L. C., Stenstrom, K. W., and Creevy, C. D. [Univ. of Minnesota, and Univ. Hosp., Minneapolis, Minn.] *Radiology,* **42**:329-345. 1944.

An analysis of 117 cases of carcinoma of the kidney gives a 5 year survival rate of 27% for the entire series. Late diagnosis is the chief obstacle to reducing the mortality rate. Nephrectomy is the treatment of choice except possibly in Wilms' tumor where irradiation seems more useful than in other types of kidney cancer. If surgery is used in Wilms' tumor, it should be in conjunction with x-ray. Preoperative x-ray may reduce the size of a mass and make it more easily removed, and postoperative x-ray may inhibit the growth of tumor cells left behind after nephrectomy. For metastases, x-ray is valuable for palliation and relief of pain. Forty-seven references are appended.—R. E. S.

Gynecomastia. A Case Associated with Mixed Tumor of Renal Origin and Testicular Atrophy. Hurxthal, L. M., and Musulin, N. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.,* **4**:38-44. 1944.

Case report with autopsy findings. The possible hormonal imbalances involved are discussed.—M. E. H.

Bilateral Renal Carcinoma. Lubert, M. [Cleveland, Ohio] *Ohio State M. J.,* **40**:657-658. 1944.

The patient, a 52 year old male, died 6 years after nephrectomy for carcinoma of the kidney and was found to have a similar tumor in the other kidney, with pulmonary metastases.—E. E. S.

Cortical Kidney Tumor—Analysis of 100 Consecutive Cases. Herger, C. C., and Sauer, H. R. [N. Y. State Inst. for Study of Malig. Dis., Buffalo, N. Y.] *Surg., Gynec. & Obst.,* **78**:584-590. 1944.

Clinical discussion.—J. G. K.

Hemangioma of the Kidney. Rives, H. F., and Pool, T. L. [Mayo Clin., Rochester, Minn.] *J. A. M. A.,* **125**:1187-1188. 1944.

A case of a rare disease is reported.—M. E. H.

Tumors of the Bladder. Coppridge, W. M. [Durham, N. C.] *Virginia M. Monthly,* **71**:500-504. 1944.

Report of 4 cases in adults and 1 case in a male child 3 years old.—M. E. H.

Extravesical Lesions Causing Bladder Neck Obstruction. Donaldson, S. W., and Ratliff, R. K. [St. Joseph Mercy Hosp., Ann Arbor, Mich.] *Radiology,* **43**:319-324. 1944.

Five cases of obstruction of the neck of the bladder, due to extravesical growths, are presented with roentgenograms. The literature is reviewed, and points in differential diagnosis are emphasized.—R. E. S.

Intrathoracic Tumors—Lungs—Heart

The Initial Neurologic and Psychiatric Syndrome of Pulmonary Growth. Meerloo, A. M. *J. A. M. A.,* **126**:558-559. 1944.

Nine persons suffering from pulmonary growths consulted a psychiatrist or a neurologist concerning their initial symptoms. Violent neuralgic pains, negative hysterical depression, and increased blood sedimentation rate are a triad of symptoms indicating organic disease and demanding immediate radiologic examination of the lungs. Diagnosis is made difficult by the initial psychologic interpretation of the symptoms.—M. E. H.

Adenoma of the Bronchus: A Clinical and Roentgenologic Study with a Report of Seven Cases. Lowry, T., and Rigler, L. G. [Univ. of Minnesota, Minneapolis, Minn.] *Radiology,* **43**:213-229. 1944.

About 6% of bronchial new growths are adenomas. This type is more frequent in women than in men, and occurs in a much younger age group than carcinoma does. It should be considered an entirely separate entity from carcinoma since the clinical course is very different. Only 2 or 3% of adenomas give metastases. The clinical features depend on the stage of the disease and the degree of obstruction. On x-ray examination the appearance may be that of atelectasis of a segment or a whole lobe. When there is extrabronchial extension, a mediastinal mass may be demonstrated. Bronchiectasis may occur, and iodized oil injection may show a "cap-shaped" defect in the bronchus. Body section roentgenography may be of great value and importance in diagnosis; bronchoscopy is necessary for final diagnosis; biopsy may be inconclusive. Adenomas bleed easily. Treatment may be by bronchoscopic removal in certain cases in which there has been little lung damage. When the lesion is treated by this means, follow-up should be maintained by roentgenography, including planography. Irradiation has had only a limited trial. The present view is that pulmonary resection is the treatment of choice in most cases because of the high incidence of local recurrence and extrabronchial extension. Lobectomy or total pneumonectomy may be necessary. Reports of 7 cases are given.—R. E. S.

Early Clinical Signs of Primary Carcinoma of the Lung. Parnell, W. [Westminster Hosp., London, England] *Tubercle,* **24**:206-211. 1943.

A statistical study of 128 cases of primary carcinoma of the lung, with reference to symptomatology. Only 66% of the patients first reported at the hospital with pulmonary signs; 34% had extrapulmonary signs and symptoms. In the latter group are included cases with enlarged liver (7%), nervous system symptoms (6%), cardiac symptoms (5%), osseous involvement (5%), and dysphagia (4%). In most of the group with pulmonary symptoms, the symptoms preceded physical signs, usually by some months, and a plea is made for early diagnosis.—C. W.

Bronchogenic Carcinoma. Schnabel, T. G. [Univ. of Pennsylvania, Philadelphia, Pa.] *J. M. Soc. New Jersey,* **41**: 402-405. 1944.

A general discussion of the means available for the early diagnosis and treatment of primary carcinoma of the lung.—M. E. H.

A Tumor Occurring in the Superior Pulmonary Sulcus. Imber, I. [Reading Hosp., Reading, Pa.] *Am. J. M. Sc.,* **207**:654-660. 1944.

Case report and discussion.—J. G. K.

Bronchiolar Origin of "Alveolar Cell Tumor" of the Lung. Herbut, P. A. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Am. J. Path.,* **20**:911-929. 1944.

Discussion, based upon a review of the literature and upon a study of 6 examples selected from a total of

90 cases of primary carcinoma of the lung. The author presents evidence to show that regenerated alveolar epithelium arises not from septal cells but from the basal cells of the bronchioles, and because of this he considers that "alveolar cell tumors" also arise from the basal cells of the bronchioles and not from septal cells.—J. G. K.

Pneumothorax Due to Metastatic Sarcoma. Report of Two Cases. THORNTON, T. F., JR., and BIGELOW, R. R. [Univ. of Chicago, Chicago, Ill.] *Arch. Path.*, **37**:334-336. 1944.

The authors failed to find reports in the recent literature of other cases of pneumothorax as a complication of metastatic tumor of the lung.—J. G. K.

Myxoma endocardii, unter dem Bilde einer Endocarditis lenta verlaufend. [Endocardial Myxoma, with a Clinical Picture Resembling That of Endocarditis Lenta.] KIRSTEIN, L. [Karolinska Inst., Stockholm, Sweden] *Acta med. Scandinav.*, **109**:77-80. 1941.

A report of a case, with autopsy findings.—M. H. P.

GASTROINTESTINAL TRACT

Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract. XIX. The Anemia of Patients with Gastric Carcinoma. OPPENHEIM, A., ABELS, J. C., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] J. A. M. A., **127**:273-276. 1945.

The authors found anemia in 64% of 122 patients with gastric cancer. The anemia varied widely with respect to the size of the red cells, but in most instances it was normochromic. "There is reason to believe that the macrocytic and normocytic anemia of these patients is not on the same basis as that of Addisonian pernicious anemia but probably is related to the associated hepatic insufficiency."—M. E. H.

Anacidity in Gastric Cancer. CHITRE, R. G., and SAMANT, V. G. [Tata Memorial Hosp., Bombay, India] *Indian M. Gaz.*, **79**:472-475. 1944.

Of 24 patients with carcinoma of the stomach, 12 showed complete gastric anacidity, 9 had very low acid secretion, and 3 had rather high acid secretion, after a 7% alcohol test meal. Gastric acidity generally showed an inverse correlation with extensiveness of the cancer, but some patients with large lesions showed higher acidity than did some patients with small lesions.—M. H. P.

Chronic Gastritis and Carcinoma of the Stomach. WARREN, S., and MEISSNER, W. A. [New England Deaconess Hosp., and Harvard Cancer Commission, Boston, Mass.] *Gastroenterology*, **3**:251-256. 1944; *reprint No. 594 of the Harvard Cancer Commission.*

Attention is drawn to the proliferative changes in mucosal epithelium, accompanying the development of exudate, when the stomach is the site of chronic inflammation. These changes are thought to be of significance as precancerous alterations.—E. E. S.

Gastric Carcinoma: Observations on Peptic Ulceration and Healing. PALMER, W. L., and HUMPHREYS, E. M. [Univ. of Chicago, Chicago, Ill.] *Gastroenterology*, **3**:257-272. 1944.

Ulcerating gastric carcinoma was found at surgery or autopsy in 4 patients. Evidence is offered that these persons had peptic ulceration of a carcinoma, rather than malignant change in a pre-existing ulcer. Ulcers in a gastric carcinoma can undergo healing despite the progression of the carcinoma.—E. E. S.

Cancer of the Stomach. BOCHAROV, A. A. *Am. Rev. Soviet Med.*, **1**:532-539. 1944.

Simultaneous resection of the stomach and omentum major to remove as many lymph nodes as possible (the Finsterer modification of the Billroth technic) is recommended as the method of choice for gastric resection when radical intervention is indicated. Statistics on 1,020 cases of gastric cancer during the years 1928 to 1938 are reported from Sklifosofski Institute.—M. H. P.

Observations on Gastric Carcinoma in Its Earliest Stages. EUSTERMAN, G. B. [Rochester, Minn.] *Wisconsin M. J.*, **43**:1138-1143. 1944.

The author reviews the significant symptoms and signs of early gastric carcinoma and points out the essentials to future progress in diagnosis and treatment.—M. E. H.

Lymphosarcoma of the Stomach: A Gastroscopic Report. PAUL, W. D., and PARKIN, G. L. [Univ. Hosp., Iowa City, Iowa] *Gastroenterology*, **3**:214-217. 1944.

A case history. Surgical excision was performed on the evidence from gastroscopic findings alone; the roentgenologist was unable to demonstrate the lesion. The appearance of the ulcerated tumor is described.—E. E. S.

Varied Clinical Manifestations of Lymphosarcoma of the Stomach. RAFSKY, H. A., KATZ, H., and KRIEGER, C. I. [Lenox Hill Hosp., and Beth Israel Hosp., New York, N. Y.] *Gastroenterology*, **3**:297-305. 1944.

No single clinical pattern is regarded as diagnostic of this condition, and the findings in 11 patients are presented to illustrate the difficulties in establishing a diagnosis. Methods of treatment are described.—E. E. S.

Primary Malignant Tumours of the Small Bowel. (A Review of 26 Cases from the Toronto General Hospital). WARREN, R. F. [Toronto Gen. Hosp., Toronto, Canada] *Canad. M. A. J.*, **51**:451-457. 1944.

A review of 26 cases of primary malignant neoplastic disease of the small bowel demonstrated that adenocarcinomas were commonest, occurring most frequently around the second part of the duodenum and duodenojejunal junction. Sarcomas were much more rare. One case of Hodgkin's disease is included. The age incidence in the group as a whole was higher than in other series; the average incidence in females was lower than in males. —M. E. H.

Malignant Carcinoid Tumors of the Small Intestine. BLUMGREN, J. E. [St. Mary's Hosp., Duluth, Minn.] *Minnesota Med.*, **27**:620-623. 1944.

Two cases of malignant carcinoid tumors of the ileum are reported, one with widespread metastases involving retroperitoneal lymph nodes, liver, lungs, spleen, ribs, sternum, and spine. Both were diagnosed post mortem. There is a possibility that some carcinoid tumors of the small bowel may be diagnosed by x-ray. Irrespective of the location, the treatment is surgery, even in the face of metastasis.—J. L. M.

Argentaffin Tumors of the Gastrointestinal Tract. Ritchie, G., and Stafford, W. T. [Univ. of Wisconsin, Madison, Wis.] *Arch. Path.*, **38**:123-127. 1944.

A case is reported of argentaffin carcinoma of the ileum with metastases in the liver and spleen.—J. G. K.

Carcinoma of Appendix. Chomet, B. [Elyria Memorial Hosp., Elyria, Ohio, and Lutheran Hosp., Cleveland, Ohio] *Am. J. Clin. Path.*, **14**:447-451. 1944.

Report of 3 cases in which morphological changes served to differentiate the growths from so-called carcinoid tumors.—J. G. K.

The Recognition and Management of Surgical Lesions of the Sigmoid and Pelvic Colon. Wolfer, J. A. [Chicago, Ill.] *Illinois M. J.*, **86**:249-255. 1944.

General discussion of preoperative and postoperative care as well as the surgical problems involved in treating polyposis, carcinoma, and diverticulitis.—M. E. H.

Clinical Features, Diagnosis, and Treatment of Carcinoma of the Colon and Rectum. Beilin, D. S. [Augustana Hosp., Chicago, Ill.] *Radiology*, **42**:539-544. 1944.

The clinical features, diagnosis, and surgical treatment of carcinoma in the various regions of the colon and rectum in 117 cases are presented.—R. E. S.

Results of Treatment of 173 Cases of Carcinoma of the Rectum. Malbin, M., and Stenstrom, K. W. [Univ. of Minnesota, and Univ. Hosp., Minneapolis, Minn.] *Radiology*, **42**:545-549. 1944.

The end results of treatment in 173 cases of carcinoma of the rectum showed a 5 year survival following surgery in 34% of 69 cases that were considered operable. The remaining 104 patients were treated by irradiation and 5% of them survived 5 years. However in this series adequate irradiation was felt to be of appreciable palliative value, and in some cases previously inoperable tumors were made amenable to surgery.—R. E. S.

Adenoma of Apocrine Sweat Glands (Hidradenoma) of the Anal Canal. Cooper, W. L., and McDonald, J. R. [Mayo Clin., Rochester, Minn.] *Arch. Path.*, **38**:155-157. 1944.

Case report, with discussion and photomicrographs.—J. G. K.

Liver and Biliary Tract

Hepatoma of the Liver with Metastasis to Bone Occurring in a Patient Known to Have Had Advanced Cirrhosis Eight Years Previously. Mensh, M., and Hanno, H. A. [Grad. Hosp. of the Univ. of Pennsylvania, Philadelphia, Pa.] *Gastroenterology*, **3**:206-213. 1944.

A case history, with photomicrographs.—E. E. S.

Malignant Hepatoma in an Infant. Litman, S. N., and Wells, A. H. [St. Luke's Hosp., Duluth, Minn.] *Minnesota Med.*, **27**:731-732. 1944.

A case of malignant hepatoma in a 2 month old white male infant is reported with a brief review of the subject.—J. L. M.

Primary Carcinoma of the Biliary Tract: Case Report. Hentel, W. [Vet. Admin., Minneapolis, Minn.] *M. Bull. Vet. Admin.*, **21**:223-226. 1944.

Primary biliary tract carcinoma, involving the pancreas and ductus choledochus by direct extension, and hepatic cirrhosis were found at autopsy.—M. E. H.

Pituitary

Acromegaly. Hurxthal, L. M., and Dee, J. F. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **3**:196-205. 1944.

Attention is called to the facial changes of early acromegaly that occur before recognizable prognathism. The importance of roentgenograms of the skull in the early diagnosis of acromegaly in patients with headache or unexplained amenorrhea is emphasized.—M. E. H..

Cancer Control and Public Health

Cancer Reporting in New York State. Levin, M. L. [N. Y. State Dept. of Health, Albany, N. Y.] *New York State J. Med.*, **44**:880-883. 1944.

Cancer reporting in upstate New York began on Jan. 1, 1940, following legislation enacted in accordance with recommendations made by the State Legislative Cancer Survey Commission. As would be expected in the reporting of any chronic disease, the largest number of reports was received during the first year. The number of reports of new cases has decreased each year by approximately 20%; this decrease is expected to continue until the number of new cases is stabilized at a level approximating the number of deaths plus the number of cured cases. In 1942, the number of new cases reported exceeded the number of deaths by 36%.

The total number of known persons with cancer alive at some time in 1942, as indicated by reporting for 1940 to 1942, was 35,378, giving an annual prevalence rate of 579 cases per 100,000 population. This is 3.6 times the mortality rate. It is the general practice for estimations of cancer prevalence to be based upon a ratio of 3 cases per death. Allowing for incompleteness of morbidity reporting, these figures for New York state indicate that this ratio is probably too low, and that prevalence may be 4 or 5 times as great as mortality. The factors of duplication of reports and the maintenance of a statewide cancer roster are discussed.

Cancer reporting has proved useful: first, in providing material for epidemiologic investigation and for evaluation of progress in cancer control; second, in public education; third, in professional education; fourth, in aiding the follow-up of cancer patients; and fifth, in the administration of public health nursing service to cancer patients. —J. L. M.

Corrections

Cancer Research, **5**:122. February, 1945. Fifth line from bottom of column 1: In *"Ztschr. f. Krebsforsch.*, **52**:319-330. 1943," for **"52"** read **"53."**

Cancer Research, **5**:249. April, 1945. Line 29 from top of column 2: For *"Xiphorus"* read *"Xiphophorus."*

Book Reviews

SUPERVOLTAGE X-RAY THERAPY. A Report for the Years 1937-1942 on The Mozelle Sassoon Supervoltage X-Ray Therapy Department, St. Bartholomew's Hospital. By Ralph Phillips, with the technical assistance of G. S. Innes. With a Foreword by The Rt. Hon. The Lord Horder. London: H. K. Lewis and Company, Ltd., 1944, vii-142 pages. Price 16s.

The Cancer Department of St. Bartholomew's Hospital (London), as a result of their evaluation of 200 kv. x-ray therapy, decided that a thorough investigation should be made of the possible advantages of higher voltages. A gift of Mrs. Meyer Sassoon provided the building and equipment, and a five year fellowship granted by the Sir Halley Stewart Trust insured that a competent radiotherapist would undertake the work and follow it through. Treatments were begun at 700 kv. in 1937 and at 1,000 kv. in 1939, and the project has been carried on with practically no interruptions, in spite of war-time hazards in London.

This book is said by its author to be an "interim report," which is true as far as evaluation of clinical results is concerned. However, more than half of the volume is given over to the results of carefully carried out physical measurements, which form a real contribution to the knowledge of the behavior of x-rays generated at approximately one million volts. These include investigations of methods of measurement of the voltage, determination of the quality and quantity of x-ray output, and tests of protection. Of particular value is a detailed study of the filtering effects of various combinations of metals. Numerous tables and graphs are given showing the relation of percentage depth dose to kilovoltage, filtration, focal-skin distance, and field size, and several isodose charts are shown.

Groups of cases treated with million-volt x-rays include inoperable carcinoma of the breast, carcinoma of the cervix uteri, malignant disease in the upper air and food passages, carcinoma of the rectum, bronchus, esophagus, and other organs. Detailed charts have been drawn up of the distribution of radiation throughout the entire irradiated region for comparison with similar charts for 200 kv. therapy. Average *tumor* doses for the million-volt rays were from 25 to 60 per cent higher than for 200 kv. rays. Because of the small number of patients in any group, it is evident that no conclusions can be drawn as yet. However, the clinical impression is that the use of the higher voltage gave better results in carcinoma of the breast, pelvic organs, and rectum, whereas in the intraoral, bronchus, and esophagus groups the findings are indeterminate. It is pointed out that in these groups there are many variable factors involved and that it is essential to have larger numbers of cases for statistical assessment.

The incidence of x-ray nausea, vomiting, and anorexia has been somewhat less with supervoltage than with 200 kv. therapy; changes in blood picture, time of healing of tissue reactions, and general convalescence about the same.

EDITH H. QUIMBY.

LYMPH NODE METASTASES. INCIDENCE AND SURGICAL TREATMENT IN NEOPLASTIC DISEASE. Grantley Walder Taylor, and Ira Theodore Nathanson. With a Foreword by Shields Warren. Illustrations and Tables. London, New York, and Toronto: Oxford University Press. 1942. xxiv + 498 pages. Price $8.00.

This book should receive consideration by every physician who is especially concerned with the treatment of cancer. It is a careful study of cancer as related to lymph node metastasis. It covers the whole field and probably represents a better than average picture of cancer as it is today when treated in a large medical center such as Boston. It would be well if we could have similar books published by men in other cities where cancer has been treated over a number of years. In this way we could get a more enlightened view as to the proper way to handle individual cases.

The book is written in three parts. Part I deals with the anatomy of the lymphatic drainage areas. Part II gives a discussion of the surgical management of lymph node metastases by regions. Part III covers the operations in lymph node areas. Ample and well selected references are given at the end of Part I and at the end of each chapter thereafter. Line drawings illustrate clearly the lymphatic pathways and the operative steps in lymph node dissections. Pertinent literature is reviewed in discussing every region as it is presented, so that the reader gets the benefit of the Boston experience as contrasted with that of others.

In Part 1 lymph node distribution is compiled and analyzed from several anatomical sources. If these line drawings could be made available in rubber stamps or chart forms for general use in hospitals, much more accurate information as to the actual dispersion of the various cancers would become available in a few years. The surgeon and the radiotherapist should be familiar with the most likely routes of spread through lymphatic channels in any given case of cancer. This book is of great assistance in helping them to decide which areas should receive special attention. From the surgeon's standpoint it is difficult to determine in some cases whether the primary growth has spread to the neighboring lymph nodes. He is then in a dilemma as to whether he should do a radical lymph node dissection on the chance that thus he might head off a wider spread of the disease later. In this book he may find considerable data to help guide him in just such a case. The rationale of lymph node operation is well covered. The diagnosis of lymph node involvement is admittedly difficult and subject to individual interpretation. Enlargement or palpability of the nodes is the most dependable guide to their involvement. Methods of physical diagnosis are given to assist in determination of the size and characteristics of the nodes. For example, bimanual palpation, with a gloved finger in the floor of the mouth and the other hand on the outside skin in the submaxillary and submental areas, will furnish more detailed information regarding node enlarge-

ment in these zones. Position of the patient and of the examiner can give more help in some situations than is generally appreciated. The biological characteristics of the various types of cancer are sufficiently known to help in predicting their behavior. Some forms rarely scatter through the lymphatic pathways; these types are considered in Part I and discussed in detail under Part II.

Cancer can be cured by total removal or destruction while it is still a local process. It can be eradicated, also, if it is confined to the regional nodes and they are accessible to surgical removal. The general rule for block dissection, removal in one piece of the original focus with the nodes that drain the area, is best illustrated by cancer of the breast. Even when the nodes are not palpable in early cancer of the breast they are assumed to have metastases present and are included in the block dissection. This is done because it has been shown that there is a considerable error in interpretation of the nodes, amounting to approximately 28 per cent. Removal of the primary focus alone and later dissection of the nodes has given less satisfactory results than when operation is done in one stage. In contrast to this attitude it has been learned by experience that in some areas eradication of the primary focus is of the utmost importance. Unless it can be satisfactorily eliminated, lymph node dissection of the areas draining it is futile. This is especially true of the intraoral group of cancers.

The skin cancers of the fingers, hands, and trunk can be treated by local removal, with careful follow-up of the regional nodes if these do not appear to be involved at the time. Prophylactic dissection can be omitted with safety only if the patients will submit to examination of the nodes at stated intervals for a 3 year period. Skin cancers of the arm, leg, and foot, however, call for prophylactic dissection of the regional nodes when the primary growth is of any considerable extent, because these lesions have been found to have a high incidence of metastasis. Differences in the handling of cancers in their varied locations are discussed in this fashion throughout Part II. The material studied and covered in this analysis is a total of 5,481 cases, with metastases in 48 per cent. Just to tabulate this material would be a problem of considerable magnitude. But throughout Part II the carcinomatous lesions are considered and evaluated from many angles. Take the tongue cancers, for example. Here they are reviewed by location: anterior third, middle third, posterior third, all thirds, and the incidence of metastases is studied; also by duration: less than 1 month, 2 to 3 months, 3 to 12 months, over 12 months; also by the size of the carcinoma: up to 1 cm., 1 to 2.5 cm., 2.5 to 3.9 cm., and 4 cm. or more; also by the gross characteristics and by the histological grading of malignancy: as to whether the lesion is primary or recurrent. The size of the lymph nodes is considered: 0 to 0.9 cm., 1 to 1.9 cm., 2 to 2.9 cm., and 3.0 cm.; the time of appearance of the metastases; the rapidity of their growth; their curability by dissection, by the time of dissection, the extent of dissection, and so forth. When the reader has finished this chapter he has a good idea of the over-all picture of tongue cancer.

The variation in lymph node metastasis given by the different forms of cancer is worth close study. The increased incidence of metastases over a mobile muscle area is an example. The surgeon and the radiologist will find much material here that will repay them for their reading.

Part III deals with the operative approach to lymph nodes in well defined regions. Rules for operability are defined. Preparation of the patient, choice of anesthesia, position on the table are given, as well as directions for the proper incisions, the things to be avoided, and the complications that may follow. This portion of the book is not emphasized and is not intended to be a guide to surgery. The authors include the types of operation that have been successful in their hands, and give their rules in these situations. Upper neck dissection, radical neck dissection, and jaw resections are discussed. Axillary lymph node dissections for breast and skin lesions, inguinal node dissections, and lymph node biopsies are given consideration.

This book can be recommended without hesitation to all students of the cancer problem. It covers a wealth of material, which is presented without bias.

JOHN MORTON.